Glial Calcium: Homeostasis and Signaling Function

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I. Introduction 100
II. Methodological Considerations 100
   A. Types of glial cells 100
   B. Experimental approaches to measure intracellular Ca$^{2+}$ 102
III. An Overview of Calcium Homeostasis in Glial Cells 102
   A. Resting intracellular Ca$^{2+}$ in glial cells 102
   B. Ca$^{2+}$-permeable channels 103
   C. Ca$^{2+}$ storage organelles, intracellular Ca$^{2+}$ release, and store-operated channels 103
   D. Ca$^{2+}$ transporters 105
   E. Intracellular Ca$^{2+}$ sensors and effectors 105
IV. Voltage-Gated Channels and Depolarization-Induced Calcium Signals 106
   A. Schwann cells 106
   B. Astrocytes 106
   C. Oligodendrocytes 107
   D. Mechanisms of glial cell depolarization 109
V. Neurotransmitter-Induced Calcium Signaling in Glial Cells 109
   A. Glutamate 109
   B. Purines and pyrimidines 115
   C. Monoamines 117
   D. y-Aminobutyric acid and glycine 118
   E. Acetylcholine 119
   F. Histamine 119
   G. Substance P 120
   H. Bradykinin 120
   I. Endothelins 120
   J. Other agonists linked to intracellular Ca$^{2+}$ regulation in glial cells 121
   K. Heterogeneity of neurotransmitter receptor expression in glial cells 124
VI. Spatiotemporal Organization of Calcium Signals 125
   A. Intracellular Ca$^{2+}$ oscillations 125
   B. Intercellular Ca$^{2+}$ waves 127
VII. Glial Calcium Signaling and Neuron-Glial Interactions 128
VIII. Glial Calcium and Brain Pathology 129
IX. Calcium Signals and Glial Function 130
X. Concluding Remarks: Calcium Signals Are a Consequence of Glial Excitability 130

Verkhratsky, Alexej, Richard K. Orkand, and Helmut Kettenmann. Glial Calcium: Homeostasis and Signaling Function. Physiol. Rev. 78: 99–141, 1998.—Glial cells respond to various electrical, mechanical, and chemical stimuli, including neurotransmitters, neuromodulators, and hormones, with an increase in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}]_{i}$). The increases exhibit a variety of temporal and spatial patterns. These [Ca$^{2+}]_{i}$ responses result from the coordinated activity of a number of molecular cascades responsible for Ca$^{2+}$ movement into or out of the cytoplasm either by way of the extracellular space or intracellular stores. Transplasmalemmal Ca$^{2+}$ movements may be controlled by several types of voltage- and ligand-gated Ca$^{2+}$-permeable channels as well as Ca$^{2+}$ pumps and a Na$^{+}$/Ca$^{2+}$ exchanger. In addition, glial cells express various metabotropic receptors coupled to intracellular Ca$^{2+}$ stores through the intracellular messenger inositol 1,4,5-trisphosphate. The interplay of different molecular cascades enables the development of agonist-specific patterns of Ca$^{2+}$ responses. Such agonist specificity may provide a means for intracellular and intercellular information coding. Calcium signals can traverse gap junctions between glial cells without decrement. These waves can serve as a substrate for integration of glial activity. By controlling gap junction conductance, Ca$^{2+}$ waves may define the limits of functional glial networks. Neuronal activity can trigger [Ca$^{2+}]_{i}$ signals in apposed glial cells, and moreover, there is some evidence that glial [Ca$^{2+}]_{i}$ waves can affect neurons. Glial Ca$^{2+}$ signaling can be regarded as a form of glial excitability.
I. INTRODUCTION

Glial intracellular calcium ([Ca\(^{2+}\)]\(_i\)), like that of other eukaryotic cells, is highly regulated; the free [Ca\(^{2+}\)] is four to five orders of magnitude less than that in the narrow system of clefts that constitutes the functional extracellular environment of the nervous system. There is, therefore, a steep electrochemical gradient favoring Ca\(^{2+}\) entry; transient cellular activation increasing Ca\(^{2+}\) permeability will lead to a transient increase in [Ca\(^{2+}\)]. In addition, there are Ca\(^{2+}\) stores within the cell that may release Ca\(^{2+}\) in response to specific intracellular chemical messengers, also increasing [Ca\(^{2+}\)]. These transient rises of [Ca\(^{2+}\)], in turn trigger or regulate various intracellular events, including metabolic processes, gene expression, and ion transport systems. Therefore, changes in [Ca\(^{2+}\)], act as an ecto-secund messenger system coordinating changes in the external environment with intracellular processes. These observations, in a wide variety of cells, have led to a general appreciation of the specific role of [Ca\(^{2+}\)], in cell signaling (for general references, see Refs. 42, 73, 147, 245, 345). Cells have developed specialized machinery to control the spatial and temporal characteristics of these Ca\(^{2+}\) signals. These include transmembrane Ca\(^{2+}\) transporters and Ca\(^{2+}\)-permeable channels, cytoplasmic buffers, and intracellular organelles that are able to accumulate, store, and release Ca\(^{2+}\).

In the nervous system, Ca\(^{2+}\) regulation has been extensively investigated and well characterized in a variety of neurons (17, 134, 290, 387, 430). These investigations demonstrate that neuronal [Ca\(^{2+}\)], participates in the control of important neuronal functions, like electrical excitability, neurotransmitter release, and long-term changes in synaptic efficacy. In parallel, but to a much lesser extent, knowledge has accumulated on the homeostasis and role of Ca\(^{2+}\) signaling in glial cells. The perception of the role of glia in brain function has changed dramatically over the last 10 years from that of a supporting glue (Greek glia is glue) with mainly trophic functions to that of a cell with dynamic interactions with neurons actively participating in nervous system function. This change occurred after the development of new techniques, like patch-clamp recording and Ca\(^{2+}\) imaging, that revealed that glial cells express a wide variety of ion channels and neurotransmitter receptors that make them able to detect and respond to neuronal activity (429, 432). Changes in glial [Ca\(^{2+}\)], have been measured under a variety of conditions where glial cells are responding to electrical, mechanical, and chemical stimuli. These fluctuations in [Ca\(^{2+}\)], appear to be a consistent response of glial cells to changes in the environment that lead to a change in glial function; they are not passive responses and, therefore, can be considered a form of glial excitability mediated by calcium. This review primarily includes recent insights into the major mechanisms involved in the control of [Ca\(^{2+}\)], and the role of changes in [Ca\(^{2+}\)], in glial signal transduction in response to neuronal activity. In addition, we consider what is known of changes in [Ca\(^{2+}\)], that affect glial function and accompany pathological processes.

II. METHODOLOGICAL CONSIDERATIONS

A. Types of Glial Cells

Glial cells (Fig. 1) are found throughout the vertebrate central nervous system (CNS) (for overview on glial cell biology, see Ref. 223). The macroglial cells, astrocytes, and oligodendrocytes are of ectodermal origin, whereas the microglial cells are thought to stem from the mesoderm. Astrocytes are probably the most diverse population of glial cells. One of their hallmarks is the expression of intermediate filament proteins, glial fibrillary acidic protein (GFAP) or S100. There is a battery of commercially available antibodies that can be used as markers to identify astrocytes. However, the expression of GFAP can vary among astrocytes and can change during development, particularly in pathological conditions. The astrocytic response to injury is marked by an increase in GFAP expression, and these cells are termed reactive astrocytes. Astrocytes in culture probably represent reactive astrocytes, since they obviously sense the strange culture environment and prominently express GFAP. Expression of GFAP is, however, not a marker for all astrocytes: Muller cells in the retina do not express GFAP under normal conditions, but only under pathological conditions. What then is the definition of an astrocyte? The answer may be that they characteristically have two contact sites, the neuronal membrane (synaptic regions in the gray matter and axons in the white matter) and the border of the CNS, either the blood system or the ventricular walls. Astrocytes can be subdivided into three major populations: radial astrocytes, fibrous astrocytes, and protoplasmic astrocytes with transition forms between these populations. Bergmann glial cells of the cerebellum are a prominent example of a radial (astrocytic glial cell). Fibrous astrocytes send a large number of processes into all directions, whereas protoplasmic astrocytes, mainly located in the gray matter, have short ramified and crimped processes (for review of astrocyte morphology, see Ref. 354).

Oligodendrocytes are the myelin-producing cells of the CNS (406). They produce myelin proteins such as myelin basic protein, proteolipid protein, myelin-associated glycoprotein, and cyclic nucleotide phosphodiesterase. Antibodies against these proteins can be used as oligodendrocyte markers. In white matter, their function seems to be well defined: they enwrap axons and form the myelin sheath. Oligodendrocytes are prominently found in white matter but can also be found in gray matter. There

are also oligodendrocytes that do not myelinate, namely, the perineuronal oligodendrocytes. At present, their functions are not known.

Microglial cells are thought to invade the brain during embryonic and early postnatal period. They stem from the monocytic lineage and thus have many common features with cells of the monocytic lineage. After invasion, they distribute equally in the brain parenchyma, and each cell seems to have a defined territory. Microglial cells are the immunocompetent cells of the CNS and can express the relevant molecules such as the major histocompatibility complex II. Under normal physiological conditions, microglial cells are in a resting state and have a small soma and fine ramified processes. After any disturbance of the nervous system, they can be activated and respond in a defined manner, converting from the resting form ultimately to a cytotoxic, phagocytic cell. This transition is graded and probably, in part, controlled by factors of the immune system, such as complement factors or cytokins (see Refs. 145, 246 for review).

All types of glial cells have been studied under a variety of conditions. There is increasing evidence that the expression of glial properties depends both on the origin of the cells and the precise experimental conditions for study. The variables are numerous and need to be precisely defined in terms of the following: 1) type of cell (including subtype where applicable), e.g., astrocyte, oligodendrocyte, Schwann cell, or microglia; 2) cellular origin, including not only the species and age of the animal but also the brain region; 3) type of preparation, in vivo, acutely prepared slice, or slice and dissociated cells in tissue culture (including time in culture and presence of other cell types), chemical environment during preparation, preservation, and experiment; and 4) experimental approaches, e.g., anatomy, electrophysiology, histochemistry, ion imaging tech-
Techniques (ion-sensitive dyes, ion probe microscopy), and molecular biology.

Given the multitude of variables, it is hardly surprising that there is little unanimity of opinion or even consistent results regarding the properties of neuroglia. The diversity of results is tending to focus on a few central problems. The control of $[\text{Ca}^{2+}]_i$, and its variation during glial responses is one of those problems.

B. Experimental Approaches to Measure Intracellular $\text{Ca}^{2+}$

Attempts to measure $[\text{Ca}^{2+}]_i$ in glial cells have paralleled those in other tissues and include the use of radioisotope tracers, $\text{Ca}^{2+}$ ion-selective electrodes, electron-probe microscopy, and in more recent time $\text{Ca}^{2+}$-sensitive fluorescent dyes (see Refs. 159, 385, 417 for review). Each of the methods has serious limitations that dictate the choice of glial preparation for study. The initial measurements of $[\text{Ca}^{2+}]_i$ were carried out in steadily growing cultures of glial cell lines (47) and primary glial cultures (283). As indicated above, glial cells change during development. Therefore, it was important to correlate the $[\text{Ca}^{2+}]_i$ measurement with the developmental stage. Thus $\text{Ca}^{2+}$ fluxes and $[\text{Ca}^{2+}]_i$ recordings were carried out along with immunostaining with stage-specific antibodies (e.g., Refs. 182, 230, 436).

Experiments on cultured cells raised the question of whether the $[\text{Ca}^{2+}]_i$ handling mechanisms remain unaltered after the cells were removed from their natural environment and maintained under artificial conditions in the absence of neurons. To solve this problem, $[\text{Ca}^{2+}]_i$ recording techniques were applied first to freshly isolated cells (e.g. Refs. 103, 131) and then to cells in acutely prepared brain slices. Initially, the technique combining patch-clamp electrophysiological recordings with $\text{Ca}^{2+}$-sensitive fluorescent dyes was applied to neurons (13, 265); later, it was used in glial cells (235, 237, 301). In these experiments, the patch-clamp whole cell configuration was employed to inject $\text{Ca}^{2+}$-sensitive probes into glial cells. This technique confines the $[\text{Ca}^{2+}]_i$, recording to a single, morphologically identified cell and allows simultaneous electrophysiological recording. However, prolonged intracellular dialysis can significantly disturb $[\text{Ca}^{2+}]_i$ regulation (235). As an alternative, $\text{Ca}^{2+}$-sensitive dye can be injected via microelectrodes into the cell of interest (104), or the slices can be incubated with membrane-permeant forms of fluorescent $\text{Ca}^{2+}$ probes (33, 239, 240, 349, 351). A major difficulty with this technique is that the background fluorescence is unknown; this may lead to a miscalculation of $[\text{Ca}^{2+}]_i$. This problem can be resolved by combining $[\text{Ca}^{2+}]_i$ measurements from cells loaded with a permeable form of the dye with subsequent intracellular dialysis. The latter helps to wash the dye from the cell of interest so that an actual background fluorescence value can be determined (240).

III. AN OVERVIEW OF CALCIUM HOMEOSTASIS IN GLIAL CELLS

The general mechanisms of $[\text{Ca}^{2+}]_i$, homeostasis are common to all eukaryotic cells (see Refs. 73, 245, 345 for review). Intracellular $\text{Ca}^{2+}$ is determined by the interaction of membrane $\text{Ca}^{2+}$ transporters and cytoplasmic calcium buffers (Fig. 2). The $\text{Ca}^{2+}$ transporters are represented by several superfamilies of transmembrane $\text{Ca}^{2+}$-permeable channels, ATP-driven calcium pumps, and electrochemically driven $\text{Ca}^{2+}$ exchangers. The resulting $\text{Ca}^{2+}$ fluxes may either deliver or remove $\text{Ca}^{2+}$ from the cytoplasm. Upon entering the cytoplasm, most $\text{Ca}^{2+}$ is trapped by $\text{Ca}^{2+}$-binding proteins; this determines the $\text{Ca}^{2+}$-buffering capacity of the cell. Calcium transporters are localized in the cell membrane (providing $\text{Ca}^{2+}$ exchange between the cell interior and exterior) and in the membrane of intracellular organelles (e.g., endoplasmic reticulum (ER), mitochondria, Golgi complex, and nucleus). The latter forms the intracellular $\text{Ca}^{2+}$ storage system (352), which actively accumulates $\text{Ca}^{2+}$. Accumulated $\text{Ca}^{2+}$ is bound to intraluminal proteins, and it can be rapidly released via intracellular $\text{Ca}^{2+}$ channels. This general scheme is applicable to all types of glial cells (429). A peculiar feature of glial cells is their high degree of heterogeneity with respect to the expression of various molecular cascades involved in $[\text{Ca}^{2+}]_i$, regulation.

A. Resting Intracellular $\text{Ca}^{2+}$ in Glial Cells

Free cytoplasmic $\text{Ca}^{2+}$ is a minor part ($<0.001\%$) of total calcium in glial cells. Most is associated with intracellular organelles (e.g., ER, mitochondria, and Golgi apparatus). Resting $[\text{Ca}^{2+}]_i$ in glial cells varies from 30–40 to 200–400 nM (see Table 1). This variation is not only among subtypes of glia, but also within the same population of cells. It may reflect method-induced artifacts or indicate the flexibility of $[\text{Ca}^{2+}]_i$, homeostasis. Most measurements were made using membrane-permeable forms of calcium indicators; thus all the problems associated with this method (uncertain calibration, dye $\text{Ca}^{2+}$ buffering, compartmentalization, and photobleaching) may contribute to the variability. Nevertheless, even in experiments performed on Bergmann glial cells in cerebellar slices (235) with careful intracellular calibration procedures, the resting $[\text{Ca}^{2+}]_i$, ranged from 30 to 200 nM. This variability did not appear to reflect cell damage, because in all cases the resting potential determined by whole cell recordings remained about normal ($–75$ to $–60\text{ mV}$).
FIG. 2. General scheme of molecular cascades involved in intracellular calcium signaling (see discussion in text). VGCC, voltage-gated Ca\(^{2+}\) channels; SOCC, stores-operated Ca\(^{2+}\) channels; PMCA, plasmalemmal Ca\(^{2+}\)-ATPase; Ca\(^{2+}\)-BP, Ca\(^{2+}\) binding proteins; InsP\(_3\), inositol 1,4,5-trisphosphate receptor/inositol 1,4,5-trisphosphate-gated Ca\(^{2+}\) channel; RyR, ryanodine receptors/Ca\(^{2+}\)-gated Ca\(^{2+}\) channel; SERCA, sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase. Intracellular Ca\(^{2+}\) sensors are as follows: CaM, calmodulin; CaM kinase, Ca\(^{2+}\)/calmodulin-dependent protein kinase; CaM AC, Ca\(^{2+}\)/calmodulin-dependent-adenylate cyclase; CaM-phosphatase, Ca\(^{2+}\)/calmodulin-dependent-protein phosphatase; Ras, p21\(^{ras}\) guanine nucleotide-binding proteins; Raf, Raf protein kinase; MEK, mitogen-activated/extracellular regulated kinase; MAPK, mitogen-activated protein kinase; IEG, immediate early genes.

B. Ca\(^{2+}\)-Permeable Channels

The initial electrophysiological surveys of glial cells of various origin (248, 249, 361; see Ref. 392 for review) did not reveal voltage-sensitive channels. With improved techniques, e.g., voltage clamping and patch clamping, the surprising finding was made that some glial cells exhibit a variety of voltage-gated ion channels that were previously believed to be present only in electrically excitable cells (23, 37, 393). Several populations of both peripheral and central macroglia were shown to express voltage-gated Ca\(^{2+}\) channels similar to those found in neurons (392). Later, it was found that Ca\(^{2+}\) influx through voltage-gated channels significantly increases [Ca\(^{2+}\)]\(_i\) in astro- and oligodendrocytes as well as in Schwann cells. However, not all glia express Ca\(^{2+}\) channels. For example, Bergmann glial cells, microglia, and certain populations of astroglia seem to lack voltage-dependent Ca\(^{2+}\) channels (192, 239, 429). Nevertheless, Ca\(^{2+}\) may enter glial cytoplasm via ligand-gated channels that are abundantly expressed in almost all glial cell subtypes (397). Finally, certain types of glial cells (e.g., retinal Müller cells or cultured astrocytes) express nonspecific cation channels that may also pass Ca\(^{2+}\) (227, 356).

C. Ca\(^{2+}\) Storage Organelles, Intracellular Ca\(^{2+}\) Release, and Store-Operated Channels

Little is known about Ca\(^{2+}\) storage organelles in glial cells. Many contain an elaborate ER (143, 354) that presumably serves as a major substrate for rapidly exchanging Ca\(^{2+}\) stores. Calcium accumulation by glial ER involves ER pumps that, like other cells, are inhibited by thapsigargin (60, 235, 237) and cyclopiazonic acid (158). *Aplysia* glial cells were found to have an unusual analog of Ca\(^{2+}\) stores, so called "gliagrana" (217, 218), which may retain an enormously high (up to 50–100 mM) Ca\(^{2+}\) concentration. The density of these gliagrana varies with fluctuations in extracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_o\)). Increases or
TABLE 1. Resting [Ca\(^{2+}\)], in glial cells

<table>
<thead>
<tr>
<th>Preparation/Cell Type</th>
<th>Method of [Ca(^{2+})], Recording</th>
<th>Resting [Ca(^{2+})], nM</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glial cell lines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glioma C6</td>
<td>Fura 2</td>
<td>75–200</td>
<td>263</td>
</tr>
<tr>
<td>Glioma C6BU-1</td>
<td>Quin 2</td>
<td>10–100</td>
<td>325</td>
</tr>
<tr>
<td><strong>Oligodendrocytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture/brain stem</td>
<td>Fura 2</td>
<td>70</td>
<td>297</td>
</tr>
<tr>
<td>Culture/cerebrum</td>
<td>Indo 1</td>
<td>5–120</td>
<td>107</td>
</tr>
<tr>
<td><strong>Astrocytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture/rat/cortex</td>
<td>Fura 2</td>
<td>34 ± 4</td>
<td>203</td>
</tr>
<tr>
<td>Culture/rat/hippocampus</td>
<td>Fura 2</td>
<td>150</td>
<td>324</td>
</tr>
<tr>
<td>Acutely isolated/rat/hippocampus</td>
<td>Indo 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture/rat/cortex</td>
<td>Fura 2</td>
<td>45–400</td>
<td>103</td>
</tr>
<tr>
<td>Culture/mouse/cortex</td>
<td>Fura 2, indo 1</td>
<td>85–220</td>
<td>156</td>
</tr>
<tr>
<td>1–2 wk</td>
<td>300–400</td>
<td></td>
<td>272</td>
</tr>
<tr>
<td>4–5 wk</td>
<td>100–200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture/cortex</td>
<td>Fura 2</td>
<td>50–160</td>
<td>140</td>
</tr>
<tr>
<td>Slice/mouse/cerebellar</td>
<td>Fura 2</td>
<td>30–200</td>
<td>235</td>
</tr>
<tr>
<td>Bergmann glial cells</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Ca\(^{2+}\)], intracellular Ca\(^{2+}\) concentration.


decreases in glial calcium depending on [Ca\(^{2+}\)]
suggest the possible involvement of glia in the regulation of [Ca\(^{2+}\)]. Similar stores have been described in frog ependymal glia and human astrocytes (143).

The major mechanism for Ca\(^{2+}\) release from internal stores involves activation of inositol 1,4,5-trisphosphate (InsP\(_3\))-gated Ca\(^{2+}\) release channels (InsP\(_3\) receptors, Refs. 34, 139). The production of InsP\(_3\), in turn, is achieved by the activation of phospholipase C (PLC) coupled via G proteins with numerous “metabotropic” plasmalemmal receptors. The nature of the InsP\(_3\) receptors subtypes in different glial cells is not known in detail. In rat cortical astrocytes and cerebellar Bergmann glial cells, only type 3 but not type 1 and 2 InsP\(_3\) receptors have been immunolocalized (453). Oligodendrocytes were reported to transiently express type 1 InsP\(_3\) receptors in a short period during the onset of myelination (98). The direct activation of InsP\(_3\) receptors by photorelease of InsP\(_3\) from caged compound was shown in cultured astrocytes (224, 382). Astrocytic InsP\(_3\) receptors appear to be substantially more sensitive to InsP\(_3\) than InsP\(_3\) receptors in Purkinje neurons; the threshold InsP\(_3\) concentration for activation of the InsP\(_3\)-gated channel in astrocytes was 0.2–0.5 μM, whereas in Purkinje neurons, it was 9 μM (224). Inositol 1,4,5-trisphosphate-induced Ca\(^{2+}\) release is involved in the majority of glial responses to neurotransmitters and neurohormones (see sect. v). The glial expression of another type of intracellular Ca\(^{2+}\) release channel, the Ca\(^{2+}\)-gated channel [or ryanodine receptor (RyR); Refs. 138, 287] is still debatable. Functional Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR), sensitive to the classical modulators ryanodine and caffeine and activated under physiological conditions, has been demonstrated only for periaxonal Schwann cells (258) and for freshly isolated Müller glial cells from salamander retina (219). In astrocytes, data on CICR are controversial; there is the one report that caffeine triggered a [Ca\(^{2+}\)], increase in cultured embryonic cortical astrocytes (158). In contrast, several observations in cultured and freshly isolated astrocytes failed to detect an obvious caffeine-triggered [Ca\(^{2+}\)], effect (60, 103). However, ryanodine and dantrolene, believed to be CICR antagonists, modulated [Ca\(^{2+}\)], responses in astrocytes (60, 253). Finally, in Bergmann glial cells, studied in cerebellar slices, caffeine and ryanodine triggered a moderate [Ca\(^{2+}\)], elevation and attenuated [Ca\(^{2+}\)], transients evoked by kainate (S. Kirischuk and A. Verkhratsky, unpublished observations). In oligodendrocytes, caffeine and ryanodine did not affect [Ca\(^{2+}\)], (238). In general (with several exceptions), glial [Ca\(^{2+}\)], appears to be insensitive to caffeine, reflecting either an absence of ryanodine receptors in glial cells or the specific expression of “brain” ryanodine receptor isoform (RyR3), which is not modulated by caffeine (149, 395). This particular ryanodine receptor subtype could be activated by a newly discovered second messenger, cyclic ADP ribose (cADPR) (141); the possible effect of cADPR on glial [Ca\(^{2+}\)], has not been investigated.

The amount of Ca\(^{2+}\) bound to internal stores may also regulate a distinct type of plasmalemmal Ca\(^{2+}\) permeability: it is widely recognized that the depletion of [Ca\(^{2+}\)], pools activates a capacitative Ca\(^{2+}\) influx. This influx is associated with the activation of specific, store-operated plasmalemmal Ca\(^{2+}\) channels (75, 188). The existence of these channels in glial cells has not been clearly shown, although there are a number of suggestions that they might be important for Ca\(^{2+}\) homeostasis in gliomas (175, 362), astrocytes (418), and microglial cells (292, 293), although at least one group (347) reported that their attempts to find the capacitative Ca\(^{2+}\) entry in cultured astrocytes failed. In microglial cells, the long-lasting activation of capacitative Ca\(^{2+}\) entry after the maximal depletion of intracellular Ca\(^{2+}\) stores has been described recently (416). Once activated, the capacitative Ca\(^{2+}\) entry pathway in microglial cells remained operative for tens of minutes, creating a steady-state [Ca\(^{2+}\)], elevation that dramatically outlasted the period of agonist action.

Mitochondria are another capacious Ca\(^{2+}\) storage site. However, their role in [Ca\(^{2+}\)], homeostasis in glial cells is little understood. The dissipation of the mitochondrial electrochemical gradient by protonophores [carbonyl cyanide m-chlorophenylhydrazone (CCCP) and carbonyl cyanide p-trifluoromethoxyphenylhydrazone] triggered Ca\(^{2+}\) release in oligodendrocytes (236, 238). However, CCCP treatment did not influence the kinetic parameters of the depolarization-triggered [Ca\(^{2+}\)], transients (238). This suggests that mitochondrial Ca\(^{2+}\) accumulation does not play an important role in calcium signal...
termination under physiological conditions. Under pathological conditions, which may substantially disturb mitochondria, glial [Ca\(^{2+}\)] \(_i\) homeostasis might be markedly affected. Alternatively, mitochondria could play an active role in Ca\(^{2+}\) signaling also under physiological conditions: in cultured oligodendrocytes, mitochondrial Ca\(^{2+}\) release/accumulation actively shaped intracellular Ca\(^{2+}\) waves and [Ca\(^{2+}\)] \(_i\) oscillations originated from InsP\(_3\)-sensitive Ca\(^{2+}\) stores (388). In cultured astrocytes, histamine-evoked [Ca\(^{2+}\)] \(_i\) oscillations were accompanied by oscillations in intramitochondrial free Ca\(^{2+}\) (209), also suggesting that mitochondrial Ca\(^{2+}\) store may play an active role in [Ca\(^{2+}\)] \(_i\), homeostasis.

The intracellular distribution of active mitochondria is different in oligodendrocyte progenitors and mature oligodendrocytes. In the former, both rhodamine-123 staining and CCCP-induced Ca\(^{2+}\) release were confined to the tips of cellular processes, suggesting that active mitochondria were concentrated in these particular areas. Conversely, in mature oligodendrocytes, mitochondria were evenly distributed (236). Presumably, the preferential localization of active mitochondria in the processes of oligodendrocytic progenitors might be important to supply energy for protein synthesis during cellular growth; it could also be important for [Ca\(^{2+}\)] \(_i\), handling in this subcellular compartment.

D. Ca\(^{2+}\) Transporters

A low [Ca\(^{2+}\)] \(_o\) and recovery from increases in [Ca\(^{2+}\)] \(_i\), produced by receptors/channels activation is provided by plasmalemmal Ca\(^{2+}\) pumps (57) and an electrochemically driven Na\(^+\)/Ca\(^{2+}\) exchanger (40). There is little information on the properties of glial Ca\(^{2+}\) pumps. However, it has been shown in oligodendrocytes that Le\(^{3+}\)-sensitive Ca\(^{2+}\)-ATPases are primarily responsible for the restoration of [Ca\(^{2+}\)] \(_i\) after a depolarization-triggered [Ca\(^{2+}\)] \(_i\) increase (238). In contrast, substantially more information is available on the expression of a Na\(^+\)/Ca\(^{2+}\) exchanger. Initial evidences concerning the existence of functional Na\(^+\)/Ca\(^{2+}\) exchange in glial cells derived from radiotracer experiments demonstrating that transmembrane fluxes of \(^{40}\)Ca\(^{2+}\) in glial cells are controlled by extracellular Na\(^+\) concentration ([Na\(^+\)] \(_o\)) (254). In several astrocytic preparations, reduction of the Na\(^+\) gradient, by lowering [Na\(^+\)] \(_o\), increased [Ca\(^{2+}\)] \(_i\), (96, 156, 158), reduced the Ca\(^{2+}\) efflux rate (178, 410), and affected the kinetics of the stimulus-evoked [Ca\(^{2+}\)] \(_i\) (84, 203). Biochemical studies (both the presence of protein and specific mRNA) revealed the expression of a heart isoform of the Na\(^+\)/Ca\(^{2+}\) exchanger in astroglial cells (156, 410). Astrocytic Na\(^+\)/Ca\(^{2+}\) exchanger was inhibited by 30-min incubation with 0.1–1 mM ascorbic acid (409) and was stimulated by sodium nitroprusside and 8-bromoguanosine 3’,5’-cyclic monophosphate (11, 411), suggesting that Na\(^-\)dependent Ca\(^{2+}\) transport in glial cells may be the target for nitric oxide. However, the relative importance of Na\(^+\)/Ca\(^{2+}\) exchanger in regulation of [Ca\(^{2+}\)] \(_i\) was questioned in several reports that showed only a minor effect of low [Na\(^+\)] \(_o\) on [Ca\(^{2+}\)] \(_i\) (103, 203). Recently, it was demonstrated (156) that a decrease in [Na\(^+\)] \(_o\), by itself, is not sufficient to increase Ca\(^{2+}\) influx via the Na\(^+\)/Ca\(^{2+}\) exchanger in astrocytes. Nevertheless, under conditions of elevated intracellular Na\(^+\) concentration ([Na\(^+\)] \(_i\)), in ouabain-treated cells, lowering [Na\(^+\)] \(_o\), produced a dramatic increase in [Ca\(^{2+}\)] \(_i\). Interestingly, a ouabainlike compound has been proposed to act as a vertebrate adrenocortical hormone (163). Thus it is possible that the physiological effect of this compound might be to increase Ca\(^{2+}\) influx via an increase in [Na\(^+\)] \(_o\).

In Bergmann glial cells in situ, Na\(^+\)/Ca\(^{2+}\) exchanger also seems to play a relatively minor role in regulating resting [Ca\(^{2+}\)] \(_i\). However, Ca\(^{2+}\) flux through the exchanger became significant under conditions of elevated [Na\(^+\)] \(_o\). Stimulation of Bergmann glial cells with kainate increased [Na\(^+\)] \(_o\), to >30 mM, which turned the exchanger in the reverse mode, providing the additional pathway for Ca\(^{2+}\) influx. This Ca\(^{2+}\) influx significantly altered both the amplitude and kinetics of the kainate-triggered [Ca\(^{2+}\)] \(_i\) signals (233).

An astrocytic Na\(^+\)/Ca\(^{2+}\) exchanger may be an important means for glia to regulate the ionic content in the interstitium. Neurons, when being electrically excited, can decrease both [Ca\(^{2+}\)] \(_i\) and [Na\(^+\)] \(_o\) in the intercellular clefts (30). Under conditions of lowered [Na\(^+\)] \(_o\), the Na\(^+\)/Ca\(^{2+}\) exchanger could reverse and supply the interstitium with Ca\(^{2+}\) by expelling it from adjacent astrocytes. Finally, Na\(^+\)/Ca\(^{2+}\) exchanger may be involved in mediating Ca\(^{2+}\) excitotoxicity under pathological conditions. In particular, reversal of Na\(^+\)/Ca\(^{2+}\) exchanger was found to play an important role in the astrocytic injury due to Ca\(^{2+}\) reperfusion after periods of Ca\(^{2+}\) depletion (a phenomenon similar to the "Ca\(^{2+}\) paradox" well described in cardiac muscle). The reperfusion-induced Ca\(^{2+}\) excitotoxicity was significantly decreased in astrocytes in which expression of Na\(^+\)/Ca\(^{2+}\) exchanger was inhibited by treatment with antisense oligodeoxynucleotides to the exchanger (282).

E. Intracellular Ca\(^{2+}\) Sensors and Effectors

After entering the cytoplasm, Ca\(^{2+}\) binds to a number of proteins that trigger various intracellular signal transduction pathways (Fig. 2, see Ref. 147 for review). Probably the best known cytoplasmic Ca\(^{2+}\) sensor is calmodulin (CaM), which regulates the functional activity of at least three broad classes of enzymes, namely, CaM-dependent protein kinases, protein phosphatases, and adenylate cyclases. The latter either interact with cytoplasmic enzymes or transfer the signal further down to the nucleus, initiating other pathways responsible for gene expression.
An alternative way to connect cytoplasmic Ca\(^{2+}\) signals and gene expression is associated with Ras proteins (small guanine nucleotide-binding proteins) which after being activated by Ca\(^{2+}\) trigger a cascade of phosphorylation events that lead to a modulation of gene expression (127). Finally, cytoplasmic Ca\(^{2+}\) signals may propagate to the nucleus, where they directly stimulate the synthesis of immediate early genes as well as structural genes. Unfortunately, little is known of the expression and role of these systems in glial cells; their characterization in glia is an important problem awaiting an experimental solution.

IV. VOLTAGE-GATED CHANNELS AND DEPOLARIZATION-INDUCED CALCIUM SIGNALS

Voltage-gated Ca\(^{2+}\) channels form an important pathway for Ca\(^{2+}\) entry in excitable cells; the latter have been found to express a variety of Ca\(^{2+}\) channels, differing in their voltage dependence, kinetics, and pharmacological properties (177, 190). Calcium channels are integral membrane proteins composed of five subunits, each playing a distinct role in channel function. The Ca\(^{2+}\) channel subunits are encoded by several gene families. The functional heterogeneity of Ca\(^{2+}\) channels arises mainly from differences in \(\alpha_1\)-subunit proteins; at least six major subtypes of \(\alpha_1\)-subunit have been cloned and characterized (177). On the basis of physiological properties and pharmacological profile, Ca\(^{2+}\) channels are classified as low-voltage-activated or T-type channels and several types of high-voltage-activated channels (code named as L, N, P, Q, and R types). Molecular classification based on the diversity of \(\alpha_1\)-subunit distinguishes six types of Ca\(^{2+}\) channels (CaCh1–6). Glial cells, although being inexcitable from the classical point of view (they are not able to generate action potentials) are capable of expressing voltage-gated Ca\(^{2+}\) channels. These have been found in several populations of macroglial cells but so far not in microglia.

A. Schwann Cells

Several early attempts to identify Ca\(^{2+}\) currents \((I_{\text{Ca}})\) in cultured Schwann cells (see Ref. 392 for review) were unsuccessful. In 1991, Amedee et al. (5) discovered that Schwann cells are able to express Ca\(^{2+}\) channels only when cocultured with neurons. Later, it was shown that expression of Ca\(^{2+}\) channels in Schwann cells could be also induced by addition of a nonhydrolyzable analog of adenosine 3',5'-cyclic monophosphate (cAMP) to the culture media (28). Whole cell patch-clamp studies of Schwann cells in organotypic cultures of mouse dorsal root ganglia (DRG) revealed voltage-dependent Ca\(^{2+}\) currents. At 10 mM Ca\(^{2+}\) outside, the \(I_{\text{Ca}}\) had an activation threshold at \(-45\) mV, current-voltage curve maximum at \(-10\) mV, and was rapidly inactivated (complete decay of current took \(\sim 150–200\) ms). The Ca\(^{2+}\) currents in cultured Schwann cells were insensitive to L-type Ca\(^{2+}\) channel modulators (nifedipine and BAY K 8644) but were blocked by 5 mM Co\(^{2+}\). In a minor cell subpopulation, a slowly decaying, nifedipine-sensitive current component was observed when using 89 mM Ba\(^{2+}\) as a charge carrier. The expression of voltage-gated Ca\(^{2+}\) channels should provide a means for generating [Ca\(^{2+}\)], transients upon Schwann cell depolarization. However, a direct attempt to measure [Ca\(^{2+}\)] elevation in Schwann cells in a similar DRG culture (267) failed to detect any measurable [Ca\(^{2+}\)] elevation in response to depolarization by 50 mM KCl.

Recently, voltage-gated Ca\(^{2+}\) channels were detected in perisynaptic Schwann cells at the frog neuromuscular junction (368). Calcium channel expression in these cells was visualized using either labeling with monoclonal antibodies against \(\alpha_1\)-\(\beta\)-subunit (monoclonal antibody 3007; Ref. 424) or fluorescent phenylalkylamine (242). Both markers clearly stained the Schwann cell membrane primarily on the processes close to transmitter release sites. The morphological observations were substantiated by confocal video imaging of [Ca\(^{2+}\)], that demonstrated that perisynaptic Schwann cells respond to high-KCl depolarization with [Ca\(^{2+}\)], transients sensitive to nimodipine (368). Thus it appears the perisynaptic peripheral glia express functional voltage-gated Ca\(^{2+}\) channels.

B. Astrocytes

MacVicar (271) first demonstrated Ca\(^{2+}\) action potentials in cAMP-treated cultured cortical astrocytes when the K\(^+\) conductance was blocked and 10 mM Ba\(^{2+}\) was added. Subsequently, similar Ca\(^{2+}\) action potentials were recorded from Muller glial cells in freshly prepared retinal slices (311), and voltage-clamp experiments on enzymatically dissociated Muller cells revealed currents carried by Ca\(^{2+}\) (311).

There are essentially two techniques to detect the presence of voltage-gated Ca\(^{2+}\) channels, either by characterizing membrane currents using electrophysiological techniques or by recording [Ca\(^{2+}\)], while activating Ca\(^{2+}\) channels with depolarization [commonly by elevating extracellular K\(^+\) concentration ([K\(^+\)]\(_{o}\)]. Calcium currents were characterized in detail in cultured cortical astrocytes (24, 71, 85, 273) and type 2 astrocytes from optic nerve (23, 25). A special treatment of cortical astrocytic cultures was necessary to record Ca\(^{2+}\) currents, namely, the addition to the culture medium of agents that increase intracellular cAMP. These include treatment with dibutyl cAMP (71, 236, 273), forskolin, isoproterenol, or certain types of sera (24, 156). Coculturing with neurons had the same effect (85). In untreated cortical astrocytes, Ca\(^{2+}\) currents were usually undetectable. In contrast, in both freshly isolated and cultured astrocytes from the optic nerve,
Ca\(^{2+}\) currents could be recorded without such pretreatment (23, 25). These results suggest that certain intracellular metabolic processes (i.e., phosphorylation) are necessary to transfer Ca\(^{2+}\) channels between “silent” and functional pools and furthermore that neurons may regulate the availability of Ca\(^{2+}\) channels in certain types of astrocytes. This also indicates that astrocytes are heterogeneous with respect to Ca\(^{2+}\) channel expression.

The parameters of astrocytic Ca\(^{2+}\) currents and the types of Ca\(^{2+}\) channels expressed vary in cells of different origin. Using a double-microelectrode voltage clamp in cultured cortical astrocytes, MacVicar and Tse (273) recorded a Ca\(^{2+}\) current that closely resembled L-type currents described in neurons. This current inactivated slowly, had a typical voltage dependence (threshold at \(-20\) mV and a maximum of the current-voltage curve at \(+10\) mV while using 10 mM Ba\(^{2+}\) as a charge carrier), was completely blocked by 1 \(\mu M\) nifedipine, and was potentiated by \(\beta\)-adrenergic agonists via increased intracellular cAMP. The amplitude of \(I_{\text{Ca}}\) in this preparation was quite substantial, reaching 4–6 nA at 5 mM extracellular Ba\(^{2+}\) and 10 nA at 10 mM extracellular Ba\(^{2+}\). In contrast, currents recorded from optic nerve astrocytes were much smaller, 200–400 pA, with 10 mM Ba\(^{2+}\) as a charge carrier (23). Furthermore, two components of \(I_{\text{Ca}}\) were recorded from optic nerve astrocytes (23, 25): inactivating (which was defined as a T-type \(I_{\text{Ca}}\) based on its kinetic and voltage dependence) and sustained, sharing properties of an L-type \(I_{\text{Ca}}\) (slow inactivation, sensitivity to low concentrations of Cd\(^{2+}\), and voltage dependence).

Similarly, small Ca\(^{2+}\) currents (<250 pA at 5 mM Ca\(^{2+}\) outside) were recorded recently from immature astrocytes in acutely prepared hippocampal slices (Fig. 3, Ref. 2). Hippocampal astrocytes in situ appear to express several types of Ca\(^{2+}\) channels as revealed by their sensitivity to various antagonists. The currents observed at voltages between \(-50\) and \(-20\) mV had parameters typical for T-type \(I_{\text{Ca}}\) (fast inactivation and sensitivity to amiloride). The \(I_{\text{Ca}}\) recorded at higher potentials were partially sensitive to nimodipine, verapamil, and \(\omega\)-conotoxin, suggesting the coexpression of L- and N-type Ca\(^{2+}\) channels.

Finally, with the employment of whole cell and perforated patch-clamp recordings, L-type Ca\(^{2+}\) currents were recorded in cultured human Muller cells (357). These currents were inhibited by dihydropyridines, but insensitive to \(\omega\)-conotoxins. The reverse transcription (RT)-polymerase chain reaction (PCR) examination of total RNA derived from cultured Muller cells revealed expression of mRNAs specific for \(\alpha_{1D}\), \(\alpha_{2}\), and \(\beta_{3}\) channel subunits, where \(\alpha_{2}\)-subunit was represented by a splice variant distinct from skeletal muscle \(\alpha_{2}\) and brain \(\alpha_{2}\)-isoforms.

An important question was to find out whether Ca\(^{2+}\) influx via voltage-gated channels alters [Ca\(^{2+}\)]. The initial attempt to resolve this problem employed fura 2 and indo 1 [Ca\(^{2+}\)], recordings from cultured embryonic (272) and neonatal (121) cortical astrocytes. In neonatal astrocytes, membrane depolarization with 25–100 mM KCl resulted in large increases in [Ca\(^{2+}\)], (up to 1 \(\mu M\)) that were inhibited by nimodipine and D-600 (121). In embryonic astrocytes, maintained in confluent culture for 4–6 wk, application of 50 mM KCl generated [Ca\(^{2+}\)], transients with an amplitude of 300–400 nM (272). The KCl-triggered [Ca\(^{2+}\)], elevation was inhibited by nifedipine and significantly potentiated by BAY K 8644, suggesting the influx was mediated by L-type Ca\(^{2+}\) channels. In astrocytes kept in a culture for only 1–2 wk, 50 mM KCl did not raise (but rather lowered) [Ca\(^{2+}\)], unless KCl was applied together with BAY K 8644. These results imply that astrocytes at early stages in culture have silent Ca\(^{2+}\) channels (272).

The variability of [Ca\(^{2+}\)] in channels in cultured cells raises questions as to the presence and function of voltage-gated Ca\(^{2+}\) entry in vivo. Freshly isolated mature hippocampal astrocytes (103) promptly respond to KCl application with [Ca\(^{2+}\)], transients (400–800 nM in amplitude in response to 50 mM KCl). Potassium chloride-induced [Ca\(^{2+}\)], transients were blocked by Co\(^{2+}\) and verapamil, but, in contrast to cultured astrocytes, they were resistant to dihydropyridines; depolarization-induced [Ca\(^{2+}\)], transients in freshly isolated astrocytes were not affected by dibutyryl cAMP. The importance of voltage-gated Ca\(^{2+}\) channels for [Ca\(^{2+}\)], regulation was further illustrated by recording [Ca\(^{2+}\)], elevation evoked by depolarizing steps in voltage-clamped fura 2-loaded astrocytes from hippocampal slices (2). Thus the data available suggest that astrocytes in vivo express voltage-gated Ca\(^{2+}\) channels and that Ca\(^{2+}\) influx through these channels substantially affects [Ca\(^{2+}\)].

C. Oligodendrocytes

Oligodendrocytes are heterogeneous with respect to the expression of voltage-gated Ca\(^{2+}\) channels. In cultures from cortex, oligodendrocytes expressed both low-voltage (T type) and high-voltage (presumably L type) Ca\(^{2+}\) currents (431, 436). The amplitudes of Ca\(^{2+}\) currents in mature oligodendrocytes were up to about 200 pA with 20 mM Ca\(^{2+}\) as a charge carrier (Fig. 3). In contrast, voltage-clamp analysis of membrane currents in cultured oligodendrocytes isolated from rat optic nerve did not reveal Ca\(^{2+}\) currents (23). In situ recordings from oligodendrocytes in a white matter preparation also failed to detect voltage-gated Ca\(^{2+}\) currents (31). It could, however, not be excluded that such channels are present in membrane patches remote from the soma such as in the paranodal loops. It is unlikely that even large current injections into the soma will lead to significant membrane depolarization at such distant regions. In support of such an uneven distribution of voltage-gated channels is an observation by Waxman and colleagues (393), who found that voltage-
FIG. 3. Ca$^{2+}$ currents in glial cells. **Left:** low-voltage-activated (LVA) and high-voltage-activated (HVA) Ca$^{2+}$ currents recorded from astrocytes in stratum radiatum of CA1 region of a hippocampal slice. Slices were prepared from 9- to 12-day-old mice. Ca$^{2+}$ currents were recorded in Na$^{-}$- and K$^{-}$-free external solutions supplemented with 1 $\mu$M tetrodotoxin; intrapipette solution contained N-methyl-D-glucamine and tetraethylammonium as major cations. **Left** trace shows family of currents evoked by different depolarizing pulses after a conditioning hyperpolarization to $-110$ mV for 1.5 s. Current apparently represents superposition of both LVA and HVA Ca$^{2+}$ currents. **Middle** trace shows HVA current in a pure form. To isolate HVA component, cells were held at $0$ mV for 1.5 s before test depolarizations. **Right** trace shows LVA current obtained as a result of subtracting HVA component from total current. Corresponding current- vs. voltage ($I$-vs. $V$) curves are shown at bottom. [From Akopian et al. (2). Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

**Right:** 2 types of Ca$^{2+}$ currents recorded from cultured oligodendrocyte. For Ca$^{2+}$ current isolation, cells were perfused with Cs$^{+}$, tetraethylammonium, and 4-aminopyridine solution while bathing in Na$^{-}$-free media; 20 mM Ba$^{2+}$ was used as a current carrier. Currents shown on left were evoked by test depolarizations to different voltages (indicated near traces) from holding potential of $0.75$ mV. On right, $I$-vs. $V$ curves for total (LVA/ HVA) Ca$^{2+}$ current and net HVA current are presented. To separate HVA current, cells were held at a holding potential of $-40$ mV. [From Von Blankenfeld et al. (436) by permission of Oxford University Press.]

gated Na$^{+}$ channels in Schwann cells are concentrated in the membrane of the paranodal loops.

The expression pattern of Ca$^{2+}$ channels undergoes considerable changes during development. Oligodendrocytic precursors from cortical cultures exhibited both T- and L-type Ca$^{2+}$ currents. (431). Channel density was very low, and whole cell $I_{\text{Ca}}$ were barely detectable (peak amplitudes <100 pA) even when Ba$^{2+}$ was used to carry current. Cultured perinatal and adult oligodendrocyte progenitors from rat optic nerve (45) had only one component of Ca$^{2+}$ current resembling the L type. In the cortical cultures, Ca$^{2+}$ currents were substantially smaller in immature oligodendrocytes/late precursors and could not be detected in young oligodendrocytes. They were readily recorded from mature cells with complex morphology. Although it was not yet possible to detect Ca$^{2+}$ channels in oligodendrocytes in situ, they were found in precursors from slices of mouse corpus callosum (31).

Despite the small amplitude of Ca$^{2+}$ currents in oligodendrocytes, Ca$^{2+}$ influx through voltage-gated channels was found to significantly increase [Ca$^{2+}$]. Depolarization of cultured oligodendrocyte precursors and mature oligodendrocytes with KCl revealed substantial [Ca$^{2+}$], increases that were sensitive to removal of [Ca$^{2+}$], inhibited by verapamil, and potentiated by BAY K 8644 (44, 45, 230, 238).

The depolarization-induced [Ca$^{2+}$] transient is spatially heterogeneous, being in general more pronounced in oligodendrocytic processes (238). Furthermore, at the early developmental stages, T- and L-type Ca$^{2+}$ channels were unevenly distributed over the cell membrane (Fig. 4). A moderate depolarization of the oligodendrocyte precursor by 20 mM K$^{+}$ led to an increase of [Ca$^{2+}$], in the processes only, whereas [Ca$^{2+}$], levels in the soma remained unaffected. A further increase in [K$^{+}$] resulted in a progressive fall in the amplitude of [Ca$^{2+}$], elevations in processes, whereas in the soma, [Ca$^{2+}$], transients became larger. Moreover, [Ca$^{2+}$], signals in processes and in the soma of oligodendrocyte precursors can be dissected pharmacologically; Ni$^{2+}$ (antagonist of low-voltage-activated Ca$^{2+}$ channels) inhibited the depolarization-induced [Ca$^{2+}$], transients only in the processes, whereas dihydropyridines preferentially affected somatic depolarization-triggered [Ca$^{2+}$], responses (238).
An uneven distribution of Ca\(^{2+}\) channels was also observed in mature oligodendrocytes; a depolarization-induced [Ca\(^{2+}\)]\(_i\) increase was mainly confined to the processes, whereas [Ca\(^{2+}\)]\(_i\) in the soma increased to a much smaller extent (238).

D. Mechanisms of Glial Cell Depolarization

The opening of voltage-gated Ca\(^{2+}\) channels requires depolarization. This depolarization might normally result from local changes in K\(^+\) concentration that accompany neuronal activity; the [K\(^+\)]\(_o\) can increase to 15 mM with intense neuronal firing (405). Such an increase in [K\(^+\)]\(_o\) was found to trigger Ca\(^{2+}\) influx via voltage-gated channels in cultured oligodendrocytes (238). Much higher levels of depolarization can be achieved under pathological conditions (e.g., spreading depression), when interstitial K\(^+\) may rise up to 80 mM (313). Alternatively, glial cells can be depolarized by the opening of ligand-gated cationic channels (see sect. V).

V. NEUROTRANSMITTER-INDUCED CALCIUM SIGNALING IN GLIAL CELLS

One of the most surprising developments in glial research over the last 25 years has been the discovery that various glial cells express a heterogeneous pattern of functional receptors to a variety of chemicals previously known to affect neurons. These include not only the classical neurotransmitters but also neuromodulators and neurohormones. Table 2 summarizes many of the experimental results that demonstrated an effect of these substances to increase [Ca\(^{2+}\)]\(_i\). Their effect results from activation of various receptors linked via several pathways to [Ca\(^{2+}\)]\(_i\), regulating molecular cascades.

A. Glutamate

Glutamate is the major excitatory neurotransmitter in the CNS of mammals, and its action is conducted via...
<table>
<thead>
<tr>
<th>Neurotransmitter/ Neuroactive Substance</th>
<th>Cell Type</th>
<th>Receptor Type</th>
<th>Mechanisms of Ca(^{2+}) Signal Generation</th>
<th>Experimental Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>Astrocytes A, P2x, P2y</td>
<td>AMPA GluRs (GluRA-D), Kainate GluRs (GluR-6)</td>
<td>Cell depolarization and Ca(^{2+}) entry via voltage-gated Ca(^{2+}) channels; Ca(^{2+}) entry via P2x receptors; InsP3-induced Ca(^{2+}) release</td>
<td>Culture (267), freshly isolated cells (288), neuromuscular junction preparation (200, 367)</td>
</tr>
<tr>
<td></td>
<td>Oligodendrocytes P2x, P2y</td>
<td>AMPA GluRs (GluRB-D), (GluR-6, 7, KAI-2) mGluR (? , minor cell population)</td>
<td>Ca(^{2+}) entry via AMPA receptors; InsP3-induced Ca(^{2+}) release (?)</td>
<td>Culture (213, 237, 407), acute brain slices (237)</td>
</tr>
<tr>
<td>ATP</td>
<td>Schwann cells A1, P2x, P2y</td>
<td>A1, P2x, P2y</td>
<td>Cell depolarization and Ca(^{2+}) entry via voltage-gated Ca(^{2+}) channels; Ca(^{2+}) entry via P2x receptors; InsP3-induced Ca(^{2+}) release</td>
<td>Culture (267), freshly isolated cells (268), neuromuscular junction preparation (200, 367)</td>
</tr>
<tr>
<td></td>
<td>Astrocytes A1, P2x, P2y, P2y</td>
<td>A1, P2x, P2y</td>
<td>Cell depolarization and Ca(^{2+}) entry via voltage-gated Ca(^{2+}) channels; Ca(^{2+}) entry via P2x receptors; InsP3-induced Ca(^{2+}) release</td>
<td>Culture (214, 228, 285, 335, 438), acute brain slices (235, 349)</td>
</tr>
<tr>
<td></td>
<td>Oligodendrocytes P2x, P2y</td>
<td>P2x, P2y</td>
<td>InsP3-induced Ca(^{2+}) release</td>
<td>Culture (213, 237, 407), acute brain slices (237)</td>
</tr>
<tr>
<td>Epinephrine, norepinephrine</td>
<td>Astrocytes α1-AR, α2-AR</td>
<td>α1-AR, α2-AR</td>
<td>InsP3-induced Ca(^{2+}) release</td>
<td>Culture (49, 114, 193, 285, 373, 382), acute brain slices (104, 239)</td>
</tr>
<tr>
<td>GABA</td>
<td>Astrocytes GABA(<em>{A}), GABA(</em>{B}) (?)</td>
<td>Cell depolarization and Ca(^{2+}) entry via voltage-gated Ca(^{2+}) channels; InsP3-induced Ca(^{2+}) release (?)</td>
<td>Culture (230)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oligodendrocytes GABA(_{A})</td>
<td>GABA(_{A})</td>
<td>Cell depolarization and Ca(^{2+}) entry via voltage-gated Ca(^{2+}) channels</td>
<td>Culture (136, 193, 285), acute brain slices (239)</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Schwann cells</td>
<td>?</td>
<td>?</td>
<td>Neurromuscular junction preparation (200)</td>
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<td></td>
<td>Astrocytes</td>
<td>M1, M2(?)</td>
<td>InsP3-induced Ca(^{2+}) release</td>
<td>Culture (92, 243, 282)</td>
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<td></td>
<td>Oligodendrocytes</td>
<td>M1</td>
<td>InsP3-induced Ca(^{2+}) release, Ca(^{2+}) entry</td>
<td>Culture (78, 213)</td>
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<td>Histamine</td>
<td>Astrocytes</td>
<td>H1</td>
<td>InsP3-induced Ca(^{2+}) release</td>
<td>Culture (136, 193, 285), acute brain slices (239)</td>
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<td>Substance P</td>
<td>Oligodendrocytes</td>
<td>?</td>
<td>?</td>
<td>Culture (213)</td>
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<td></td>
<td>Astrocytes</td>
<td>NK, NK</td>
<td>?</td>
<td>Culture (193, 278, 281)</td>
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<tr>
<td>Bradykinin</td>
<td>Schwann cells</td>
<td>?</td>
<td>?</td>
<td>InsP3-induced Ca(^{2+}) release</td>
</tr>
<tr>
<td></td>
<td>Astrocytes</td>
<td>B2</td>
<td>InsP3-induced Ca(^{2+}) release, Ca(^{2+}) entry</td>
<td>Culture (152, 399)</td>
</tr>
<tr>
<td>Endothelin</td>
<td>Astrocytes</td>
<td>ET(<em>{A}), ET(</em>{B})</td>
<td>InsP3-induced Ca(^{2+}) release, Ca(^{2+}) entry via voltage-gated (L) Ca(^{2+}) channels (?)</td>
<td>Culture (213, 278)</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Schwann cells</td>
<td>5-HT2a</td>
<td>InsP3-induced Ca(^{2+}) release (?)</td>
<td>Culture (457)</td>
</tr>
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<td></td>
<td>Astrocytes</td>
<td>5-HY2c</td>
<td>?</td>
<td>Culture (64, 193, 316, 425)</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>Astrocytes</td>
<td>?</td>
<td>InsP3-induced Ca(^{2+}) release</td>
<td>Culture (99)</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>Astrocytes</td>
<td>V1</td>
<td>InsP3-induced Ca(^{2+}) release</td>
<td>Culture (168, 193, 210)</td>
</tr>
<tr>
<td>Neuropeptide Y</td>
<td>Astrocytes</td>
<td>?</td>
<td>Depolarization and Ca(^{2+}) entry via voltage-gated Ca(^{2+}) (L) channels</td>
<td>Culture (151)</td>
</tr>
<tr>
<td>Complement fragments</td>
<td>Astrocytes</td>
<td>?</td>
<td>InsP3-induced Ca(^{2+}) release, capacitative Ca(^{2+}) entry</td>
<td>Culture (292)</td>
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<td>Microglia</td>
<td>C5a, C3a receptors</td>
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activation of highly diversified families of ionotropic and metabotropic receptors. The ionotropic glutamate receptors (GluRs) are ligand-gated cationic channels assembled from five subunits. There are three groups of ionotropic GluRs (according to their pharmacological properties), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainate, and N-methyl-d-aspartate (NMDA) (180). The recent advances in recombinant DNA techniques have given a precise characterization of the GluRs subunit structure and revealed the molecular determinants of GluRs permeability, gating mechanisms, and agonist specificity (180, 207). The GluRs subunits A, B, C, and D (or 1–4) form AMPA-sensitive receptors, GluR5, -6, and -7, and subunits denoted as KA1 and KA2 assembled to form kainate-preferable GluRs. Finally, the NMDA-sensitive GluR subfamily is formed by NMDA R1 and NMDA R2A-D subunits (295). Various GluRs subunits can be differentially assembled forming homo- or heteromeric channels that bear different functional properties. The subunit structure of the GluRs determines their Ca\(^{2+}\) permeability, with a crucial role for the GluR B subunit; channels containing GluR B subunit are almost impermeable to Ca\(^{2+}\), and those lacking this subunit in the channel pentamer are highly Ca\(^{2+}\) permeable (52, 146).

Metabotropic glutamate receptors (mGluRs) also comprise a distinct gene family of at least eight members (mGluRs 1–8); the mGluRs belong to the so-called seven-membrane spanning domains receptors (307, 348). The mGluR1 and -5 are coupled (via G proteins) with PLC, being thus the activators of the InsP\(_3\)-mediated intracellular signaling pathway; other mGluRs are connected with adenylyl cyclases.

1. Schwann cells

Initial suggestions that GluRs might be expressed by Schwann cells came from experiments on squid axons in which nerve stimulation appeared to trigger a hyperpolarization of periaxonal Schwann cells that could be mimicked by glutamate (434) and blocked by a glutamate antagonist (2-amino-4-phosphonobutyrate) (260, 261) or internal administration of the Ca\(^{2+}\) chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (262). The latter observation suggested the effect involved an increase in [Ca\(^{2+}\)]. At the same time, electrophysiological experiments appeared to reveal NMDA-mediated depolarizing responses in squid Schwann cells (117, 118). These potentially interesting observations have not been confirmed in other molluscan species and require confirmation with more modern techniques.

In mammalian peripheral nerve, Schwann cells were intensively stained by specific antibodies against GluR B and D (97), suggesting the possible existence of functional AMPA receptors. However, their link to changes in [Ca\(^{2+}\)], remains unknown. Only a minor fraction (<10%) of freshly dissociated Schwann cells from neonatal rat sciatic nerves responded to glutamate with an increase in [Ca\(^{2+}\)] (267). Thus the involvement of glutamate receptors in [Ca\(^{2+}\)]\(_i\) control in Schwann cells is still unclear and needs further examination.

2. Astrocytes

The GluRs were probably the first neurotransmitter receptors found in astroglia. In 1981, Orkand et al. (328) found that glutamate depolarized glial cells in optic nerve
preparation of Necturus; later, in 1984, Kettenmann et al. (221) and Bowman and Kimelberg (46) showed that excitatory amino acids (glutamate, aspartate, and kainate) directly depolarized cultured astrocytes. An alternative mechanism for a glutamate-dependent depolarization is stimulation of the electrogenic Na⁺-dependent glutamate transporter by an increase in external glutamate (12, 212). However, the effectiveness of kainate, a specific agonist of kainate/AMPA receptors that is not transported by glutamate transporter, already implied the presence of glutamate receptors. This was substantiated by the observation that the glutamate effect is mediated by changes in intracellular phosphoinositide turnover and transmembrane fluxes of ⁴⁰Ca²⁺ (336). More recently, microfluorimetric techniques revealed that glutamate induces complex changes in [Ca²⁺]i, characterized by distinct spatiotemporal features often in the form of intracellular waves and oscillations. These glutamate-triggered Ca²⁺ responses were mediated by both transmembrane Ca²⁺ entry and intracellular Ca²⁺ release, indicating the involvement of several types of GluRs (225).

A) AMPA/KAINATE IONOTROPIC GLUTAMATE RECEPTORS.

1) Cultured cells. The initial observations of excitatory amino acid-induced depolarization of astrocytes were substantiated in voltage-clamp experiments that demonstrated that glutamate, quisqualate, AMPA, and kainate, but not NMDA triggered Na⁺/K⁺ currents in cultured astrocytes from cerebrum and cerebellum (394, 449). These currents were blocked by the specific antagonists of AMPA/kainate receptors, 6-cyano-7-nitroquinazoline-2,3-dione (CNQX) and 6,7-dichloro-3-hydroxy-2-quinoxalinecarboxylic acid (394, 448). Single-channel recordings revealed that glutamate-activated currents had several conductance levels, and their kinetic properties were similar to those for AMPA/kainate receptors in neurons (422, 448). Thus experimental evidence suggests that astrocytes are endowed with AMPA/kainate GluRs.

Recordings of [Ca²⁺]i with fura 2-based microfluorimetry demonstrated that kainate and AMPA (112, 154) raised [Ca²⁺]i, in cultured cerebral, hippocampal, and cerebellar astrocytes. This [Ca²⁺]i rise depended on [Ca²⁺]o, and was blocked by CNQX. Similar AMPA- and kainate-evoked [Ca²⁺]i transients were observed in retinal Müller cells (437). In mixed cultures from neonatal rat brains, the CNQX-sensitive AMPA/kainate-triggered [Ca²⁺]i transients were mostly confined to type 1 astrocytes (204), suggesting differential expression of GluRs in astroglia. Thus activation of AMPA/kainate receptors in astrocytes promotes Ca²⁺ influx that might result either from depolarization-triggered activation of voltage-gated channels or from direct Ca²⁺ influx through GluRs.

Initial experiments on glutamate-induced Ca²⁺ signaling in glial cells coincided with the detection of Ca²⁺ permeability of AMPA/kainate GluRs in neurons (179, 191) and the subsequent discovery of its molecular basis (52, 207). The latter findings stimulated the search for Ca²⁺-permeable GluRs in glia. Initially, it was found that Co²⁺, which is thought to substitute for Ca²⁺ as a permeable ion through AMPA/kainate GluRs, permeates and can be stained within cerebellar type 2 astrocytes, suggesting the expression of Ca²⁺-permeable GluRs (355). High-Ca²⁺ permeable AMPA receptors were described in cultured Bergmann glial cells, and simultaneously, in situ hybridization indicated that these cells lack the GluR B subunit (53). These findings were consistent with the hypothesis that the presence of GluR B in the channel heteromer inhibits Ca²⁺ permeability (146). No GluR B subunit mRNA was found to be associated with the expression of GluR A (mainly) and GluR C subunits in glial cells (presumably astrocytes) from rat optic nerve (205). Northern blot analysis of mRNA for AMPA GluRs subtypes performed on primary cultured astrocytes revealed that cells isolated from the brain stem express predominantly GluR D specific mRNA (81).

Cortical astrocytes and astrocyte progenitors expressed GluR B mRNAs as well as mRNAs encoding GluRs A, C, D and GluR6 subunits, as demonstrated by both Northern blots (81) and RT-PCR technique (182). Despite the apparent presence of GluR B subunit, these cells respond to kainate with large [Ca²⁺]i transients (182). These transients were not modified by Na⁺ removal from the bath and were not attenuated when intracellular Ca²⁺ stores were blocked with thapsigargin. Furthermore, stimulation of astrocytes by kainate, when external Ca²⁺ was replaced by Co²⁺ and [Na⁺]o was removed, caused fast quenching of fura 2 signals, indicating that Co²⁺ entered the cell via kainate-activated channels. These results suggested that [Ca²⁺]i elevation in cortical astrocytes resulted mainly from Ca²⁺ entry via AMPA/kainate receptors (182). However, kainate-induced currents, measured under voltage-clamp conditions in the same cells, were drastically decreased (~40 times) in the absence of Na⁺, suggesting a low Ca²⁺ permeability of the receptor. Similarly, glial cells (most likely immature astrocytes) acutely isolated from the hippocampal CA1 stratum radiatum region, exhibited a low or intermediate Ca²⁺ permeability, as determined by potential-dependent characteristics of kainate-induced ionic currents (378). Nevertheless, even these small Ca²⁺ currents via low-Ca²⁺ permeability AMPA/kainate receptors are able to appreciably increase [Ca²⁺]i, in astrocytes. This may indicate a low Ca²⁺ buffer capacity in cortical astrocytes.

2) In situ preparations. Initial evidence for the expression of functional GluRs in glial cells in situ was revealed by microelectrode recordings from astrocytes in rat hippocampal slices and amphibian optic nerve; application of glutamate depolarized these astrocytes (414, 440). Later, patch-clamp recordings revealed AMPA-, kainate-, and quisqualate-induced ionic currents sensitive to CNQX in rabbit retinal astrocytes from in situ preparation.
FIG. 5. Stimulation of ionotropic glutamate receptors (GluRs) in Bergmann glial cell in cerebellar slices triggers Ca\(^{2+}\) influx. A: pseudocolor image of a fura 2-loaded Bergmann glial cell (scheme of experiment is shown in inset on left) in control conditions and upon extracellular application of 100 \(\mu\)M kainate. Note preferential increase in \([Ca^{2+}]_i\) in cell processes. B and C: \([Ca^{2+}]_i\) and membrane current traces recorded from same cell. Both removal of extracellular \([Ca^{2+}]_i\) (B) and blockade of ionotropic GluRs by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 \(\mu\)M) inhibited kainate-induced currents and \([Ca^{2+}]_i\) elevation, suggesting a key role of \(Ca^{2+}\) influx. \(F_{340/380}\), fluorescence ratio at 340/380 nm. (From T. Müller and H. Kettenmann, unpublished data.)

Subsequently, kainate-induced cationic currents associated with significant \(Ca^{2+}\) entry (as measured by fura 2 microfluorimetry) were recorded from Bergmann glial cells in acutely prepared cerebellar slices (Fig. 5, Ref. 301). The high \(Ca^{2+}\) permeability of AMPA/kainate receptors in Bergmann glial cells coincides with the absolute absence of GluR B mRNA as determined by single cell RT-PCR (146, 207); Bergmann glial cells appear to be the only cell in the brain that completely lacks the GluR B subunit. The relative permeability ratio \(P_{Ca}/P_{Na}\) determined for AMPA/kainate GluRs in Bergmann glial cells was 2.8 (207). The \(Ca^{2+}\) permeability of AMPA/kainate receptors expressed in glial cells was substantially modified during development; the \(P_{Ca}/P_{Na}\) was found to be downregulated from 2.1 to 0.9 during the second postnatal week in hilar progenitor cells studied in acutely isolated hippocampal slices (14).

In immature astrocytes from hippocampal slices studied under voltage-clamp conditions, glutamate evoked cationic currents with a pharmacology typical of AMPA/kainate receptors (398). Single-cell RT-PCR experiments revealed the coexpression of GluR B and GluR D subunits (397), implying a low \(Ca^{2+}\) permeability of heteromeric AMPA GluRs. Indeed, electrophysiological analysis revealed a low \(P_{Ca}/P_{Na}\) (0.12–0.24) for AMPA receptors in hippocampal astrocytes (378). However, kainate was able to generate prominent \([Ca^{2+}]_i\) transients in these cells in acute hippocampal slices (199). The kainate-triggered \([Ca^{2+}]_i\) rise likely reflects \(Ca^{2+}\) influx via GluRs, since both voltage-clamp and microfluorimetric experiments failed to detect appreciable amounts of \(Ca^{2+}\) entry via voltage-gated \(Ca^{2+}\) channels. Thus, even a small \(Ca^{2+}\) influx through low-\(Ca^{2+}\) permeable AMPA receptors might generate significant \(Ca^{2+}\) signals in astrocytes. Similarly,
GFAP-positive mature rat hippocampal astrocytes responded with a [Ca\(^{2+}\)]\(_i\) increase to kainate and AMPA; these [Ca\(^{2+}\)]\(_i\) responses were blocked by CNQX (350). Furthermore, neither verapamil nor nifedipine prevented kainate-induced [Ca\(^{2+}\)]\(_i\) transients, suggesting that Ca\(^{2+}\) influx occurred through GluRs.

Finally, [Ca\(^{2+}\)]\(_i\) recordings using confocal microscopy demonstrated glutamate-evoked [Ca\(^{2+}\)]\(_i\) transients in periaxonal glial cells (presumably astrocytes) in optic nerves (247). The [Ca\(^{2+}\)]\(_i\) rise in periaxonal glial cells in the optic nerve was also elicited by 1-aminocyclopentane-1,3-dicarboxylate [(1S,3R)-ACPD] and AMPA and was partially sensitive to the AMPA antagonist 6,7-dinitroquinoxaline-2,3(1H,4H)-dione, suggesting the functional expression of both ionic- and metabotropic GluRs in optic nerve glial cells.

B) NMDA RECEPTORS. In one study, an NMDA-induced [Ca\(^{2+}\)]\(_i\) rise was observed (1) in some cultured spinal cord astrocytes. In most other studies of cultured astrocytes, examined with both electrophysiological techniques (384, 422, 448, 449) and Ca\(^{2+}\)-sensitive fluorescent dyes (182), NMDA caused no effects on membrane permeability and [Ca\(^{2+}\)]\(_i\). A notable exception was experiments on radial glia; studies of cultured retinal Muller cells found that glutamate promoted their proliferation via activation of receptors with NMDA receptor (NMDAR) pharmacology (421). Subsequent electrophysiological observations detected NMDA-evoked currents in Muller cells (358). In another type of radial glia, cerebellar Bergmann glial cells, voltage-clamped in cerebellar slices, bath application of 1 mM NMDA evoked tiny (~30–60 pA) currents (300). In situ hybridization revealed a significant level of expression of NMDAR1 and -2B subunits mRNA in these cells (266), although the exact composition of NMDA receptors assembled in the membrane remains unknown. The NMDA-activated currents in Bergmann glial cells were not associated with measurable changes in [Ca\(^{2+}\)]\(_i\) (300). Similarly, fura 2-based experiments failed to detect any [Ca\(^{2+}\)]\(_i\) changes in retinal Muller cells challenged with NMDA (437). Finally, NMDA-activated currents have been observed in neocortical protoplasmic astrocytes (229) and in a small population of hippocampal astrocytes (398). The question of whether NMDA can induce [Ca\(^{2+}\)]\(_i\) increases in astrocytes remains unclear. Using confocal video imaging of hippocampal astrocytes, Porter and McCarthy (350) observed [Ca\(^{2+}\)]\(_i\) transients in response to bath applications of NMDA; however, these Ca\(^{2+}\) responses could have been triggered indirectly by activation of neuronal terminals in the hippocampal slices with subsequent release of glutamate and activation of non-NMDAR in glial cells.

C) METABOTROPIC GLUTAMATE RECEPTORS. Another important route for generating Ca\(^{2+}\) signals in astroglia is associated with the activation of mGluRs and subsequent Ca\(^{2+}\) release via InsP\(_3\)-gated intracellular Ca\(^{2+}\) channels. Biochemical investigations clearly demonstrated an increase in intracellular InsP\(_3\) level in glutamate-treated astroglial cells (289, 336). The mGluR-mediated Ca\(^{2+}\) signaling is widespread in astrocytes. A majority of [Ca\(^{2+}\)]\(_i\) recordings from cultured astrocytes (1, 83, 129) suggest that a substantial part of the glutamate-induced [Ca\(^{2+}\)]\(_i\) elevation persists in Ca\(^{2+}\)-free extracellular solutions, indicating that the Ca\(^{2+}\) comes from internal stores. These intracellular Ca\(^{2+}\) responses were mimicked by a specific agonist of mGluRs (1S,3R)-ACPD (129), pointing to the involvement of the Ghr-R-InsP\(_3\) signal transduction chain. The nature of astrocytic mGluRs is still unclear; the expression of mGluR3 and mGluR5 only was found in glia (370, 413). Strong mGluR5-dependent immunostaining was found in astrocytic processes in hypothalamus in situ (426); these processes surround complex synapses; mGluRs may well be exposed to glutamate during synaptic activity. The importance of mGluRs in triggering Ca\(^{2+}\) responses in astroglia was also confirmed by in situ experiments. The [Ca\(^{2+}\)]\(_i\) transients mediated via mGluRs were found in both astrocytes in hippocampal slices (350) and Bergmann glial cells in cerebellar slices (232). In the latter, the expression of both mGluR1 and mGluR5 was determined by using single-cell RT-PCR (Kirchhoff, Matiash, F. Kirchhoff, H. Ketenmann, and A. Verkhratsky, unpublished observations). The relative expression of mGluR1/mGluR5 receptors could be important for the shaping of glutamate-evoked [Ca\(^{2+}\)]\(_i\) transients. It has been demonstrated recently that transfected cells that express exclusively mGluR5 respond to glutamate with [Ca\(^{2+}\)]\(_i\), oscillations, whereas cells expressing mGluR1 had single-peak [Ca\(^{2+}\)]\(_i\) responses (216). The question of whether mGluR1/mGluR5 might be important for determining the kinetic characteristics of glial [Ca\(^{2+}\)]\(_i\) responses remains to be clarified.

3. Oligodendrocytes

Electrophysiological studies of cultured immature oligodendrocytes and their progenitors (44, 142, 334) as well as oligodendrocyte progenitors in corpus callosum slices (32) found glutamate-, AMPA-, and kainate-triggered ionic currents that were blocked by CNQX, suggesting AMPA/kainate ionotropic receptors were stimulated. Indeed, Northern blots revealed the expression of mGluRs B, C, and D, GluR6 and -7, and KA1 and KA2 mRNAs in cells of the oligodendrocyte lineage (334). High expression of GluR B subunit implies a low Ca\(^{2+}\) affinity of oligodendrocyte AMPA/kainate channels.

Intracellular Ca\(^{2+}\) recordings from oligodendrocyte cultures also showed increases after application of glutamate and its agonists. According to Borges et al. (44), cytoplasmic Ca\(^{2+}\) increases after the activation of AMPA/kainate receptors in oligodendrocyte precursors resulted mainly from Ca\(^{2+}\) influx via voltage-gated channels. Other authors (181, 182, 288) suggest that Ca\(^{2+}\) influx through
GluRs can be also involved. Minor populations of cultured oligodendrocytes also exhibited mGluRs (181). The expression of AMPA/kainate GluRs in cells of the oligodendrocyte lineage was found to be developmentally downregulated. Mature oligodendrocytes lost the ability to respond to glutamate by activation of membrane currents (44). In another study, the upregulation of GluR B subunit abundance during transition of O2-A and CG-4 progenitors into oligo- or astrocytes was demonstrated (288). Likewise, in optic nerves, quisqualate-stimulated Co2+ uptake (which is believed to reflect Co2+ entry through Ca2+-permeable AMPA receptors) only in O-2A progenitor cells but not in mature glia (137).

B. Purines and Pyrimidines

Adenosine 5’-triphosphate, adenosine, and related substances control a number of important physiological reactions and act as neurotransmitters in the peripheral nervous system and CNS (54, 102, 133). In recent years, clear evidence that ATP acts as an excitatory neurotransmitter in the CNS has been obtained (108, 109), and pharmacological and molecular characterization of ATP and adenosine receptors (named purinoreceptors) was achieved. Purinoreceptors are represented by a broad family of proteins classified into two major groups (88, 133): 1) adenosine receptors (or P1 purinoreceptors code-named also as A1-A3 receptors) coupled mainly with adenylate cyclases as well as with PLC (A1 receptors) and 2) receptors for ATP and related nucleotides known as P2 purinoreceptors. On the basis of pharmacological properties, the P2 purinoreceptor family is subclassified into two groups: ionotropic receptors (P2x and P2y) and metabotropic receptors (P2y, P2u, P2t, and P2a). Advances in molecular cloning extended this classification by showing that purinoreceptors are encoded by two distinct gene families (55). The family of P2x receptors comprises several subtypes (labeled P2x1-7) of ligand-gated ionic channels with a unique two transmembrane domain topology; the members of P2x family differ in their ion selectivity and gating properties. Cloned P2x receptors also belong to the P2x gene family, and they are codenamed as P2x subtype; P2x7 receptors are large transmembrane pores that are activated in fact by a tetraanionic form of ATP (ATP4−) and may pass molecules with a molecular mass up to 1 kDa. Cloned metabotropic receptors are classified as P2y family, being represented by seven members (P2y1–P2y7). They all are similar to other G protein-linked metabotropic receptors by their seven-transmembrane domain structure and are often associated with PLC and hence InsP3 turnover. The P2y1 receptor pharmacologically matches P2x subtype, P2y2-P2x2-P2y3 probably corresponds to P2x receptor; P2y4–7 are not yet assigned with known subtypes.

Purinoreceptors are unusually widely distributed among glial cells. A majority of glial cells studied (including peripheral glia, macro- and microglia from the CNS) appear to express various types of purinoreceptors. In many cases, activation of glial purinoreceptors leads to increases in [Ca2+]i.

1. Schwann cells

An ATP-triggered increase in [Ca2+]i, was first demonstrated by Jahromi et al. (200) who monitored [Ca2+]i in perisynaptic Schwann cells at the frog neuromuscular junction; they also suggested that Schwann cells respond with a [Ca2+]i increase to synaptically released ATP after electrical stimulation of the axon. The calcium transients recorded by Jahromi et al. (200) were confined to perisynaptic Schwann cells only; the myelinating Schwann cells situated periaxonally did not respond to ATP. Intracellular Ca2+ transients induced by ATP and mediated via activation of P2y receptors were found in a majority of freshly dissociated Schwann cells from neonatal rat sciatic nerve (267) as well as in periaxonal sciatic nerve Schwann cells studied in situ (268). Interestingly, P2y1-driven [Ca2+]i responses in neonatal rat Schwann cells were significantly downregulated when cells were maintained in culture for several days. However, these responses were restored either by the addition of membrane permeable cAMP analogs (dibutyryl cAMP or 8-bromo-cAMP) to the culture media (267) or coculturing glial cells with DRG neurons (267, 268). In Schwann cell cultures obtained from adult rats and rabbits, the predominant receptor type was P2y9 (9), suggesting that the expression of particular purinoreceptor subtypes might undergo developmental changes. Finally, an analysis of purinoreceptors at the frog neuromuscular junction perisynaptic Schwann cells revealed metabotropic P2y adenosine and P2y purinoreceptors that both induce intracellular Ca2+ release and involve pertussis toxin (PTX)-sensitive G protein transduction pathways, as well as ionotropic P2x receptors that act via membrane depolarization and Ca2+ entry through plasma-membrane L-type Ca2+ channels (367). Voltage-clamp experiments on cultured mouse DRG Schwann cells also revealed the existence of an ATP-triggered cationic current, sensitive to P2 purinoreceptor antagonist suramin and carried to certain extent by Ca2+ (4). The unusually high concentrations of ATP required to activate this current (dissociation constant ~8.4 mM) suggests P2x receptors are involved. All experimental data available indicate that Schwann cells express purinoceptors linked to the regulation of [Ca2+]i, and this might be an important pathway for neuronal-glial interaction in the peripheral nervous system. This interaction is presumably accomplished via either synaptic or nonsynaptic release of purines after axonal electrical activity.

2. Astrocytes

Numerous experiments on cultured astrocytes demonstrate that they express both adenosine (P1) and P2 purinoreceptors involved in the regulation of [Ca2+]i.
A) ADENOSINE (P_1) PURINORECEPTORS. Apart from A_1 and A_2 receptors present in astroglia, which either inhibit (A_1) or stimulate (A_2) adenylate cyclase, recent experiments demonstrated that adenosine may also increase \([Ca^{2+}]_i\) in astrocytes (96, 335). This \([Ca^{2+}]_i\) increase originates from intracellular \(Ca^{2+}\) stores. It was mimicked by selective A_1 receptor agonist N\(^6\)-cyclopentyladenosine and inhibited by A_2 antagonist 8-cyclopentyl-1,3-dipropylxanthine (335). Additionally, stimulation of A_1 receptors was reported to potentiate the \([Ca^{2+}]_i\) mobilizing activity of other neuroactive substances, including histamine (335), substance P (96) and the mGluR agonist (1S,3R)-ACPD (324). The ability of adenosine to induce \(Ca^{2+}\) mobilization from internal stores was further substantiated in video-imaging experiments on astrocytes in acutely isolated rat hippocampal slices (349). The agonist/antagonist sensitivity of adenosine-induced \([Ca^{2+}]_i\) transients in slice preparations did not match the pharmacological profile for A_1, A_2 or A_3 receptors; the possible involvement of A_3 receptors (452) was suggested. The \([Ca^{2+}]_i\) mobilization mediated through adenosine receptors was reported to trigger nitric oxide release from cultured astrocytes (202).

B) P_2 PURINORECEPTORS. Many studies of cultured astrocytes have shown that ATP increases \([Ca^{2+}]_i\). Both ionotropic and metabotropic P_2 purinoreceptors appear to participate in this response. The ATP-stimulated \(Ca^{2+}\) influx was found first in experiments measuring \(46Ca^{2+}\) uptake in astrocytes (309). This effect of ATP was inhibited by LaCl_3, suggesting \(Ca^{2+}\) entered via plasmalemmal \(Ca^{2+}\) channels. Subsequent current- and voltage-clamp studies found that ATP depolarized cultured cerebral astrocytes by triggering an inward cationic current, most likely due to stimulation of P_2x receptors (274, 438). This cationic current may activate voltage-gated \(Ca^{2+}\) channels (438). Alternatively, P_2x receptors may have an appreciable \(Ca^{2+}\) permeability. Experiments with other cells suggest that several P_2x receptor subtypes have a significant \(Ca^{2+}\) permeability (423). The \(Ca^{2+}\) current can be up to 6.5% of the total under physiological conditions (369). Furthermore, astrocytic calcium signaling could be mediated through P_2x ionotropic receptors as demonstrated on primary cultured rat cortical astrocytes (19).

Another important route for \([Ca^{2+}]_i\) increases in astrocytes results from metabotropic P_2 receptor stimulation. Studies on purinoreceptor agonists suggest that the predominant type of metabotropic ATP receptor expressed by astroglia is the P_2y (214, 285, 374, 375), although there are reports that primary cultured cortical (50, 338) and dorsal spinal cord (176) astroglial cells express P_2x receptors linked to InsP_3 turnover and \(Ca^{2+}\) mobilization from internal stores. Likewise, P_2a receptors linked to \([Ca^{2+}]_i\) increase were found in enteric glial cells (226), which are similar to astrocytes with respect to their morphology and GFAP expression. Intracellular \(Ca^{2+}\) increases and \([Ca^{2+}]_i\) oscillations mediated by P_2a receptors have also been described in astrocyte-derived glioma cells (87). The coexpression of both P_2a and P_2x receptors were demonstrated for cultured cortical astrocytes from neonatal rat (228). That was shown by injecting the Xenopus oocytes with mRNA collected from confluent astrocytic cultures. The injected oocytes expressed both P_2a and P_2x receptors as demonstrated by their pharmacological profile.

Activation of P_2a receptors leads to an increase in intracellular InsP_3 in cultured cortical astrocytes (214, 338, 340), resulting in InsP_3-driven \(Ca^{2+}\) release from internal stores. The involvement of PLC controlled InsP_3 production and activation of InsP_3-gated ER \(Ca^{2+}\) channels was directly demonstrated in experiments on cultured astrocytes from rat cerebral cortex and spinal cord. In both preparations, an increase in intracellular InsP_3 [by either flash photolysis (382) or intracellular dialysis (375)] mimicked the effects of ATP. The sensitization of InsP_3 receptors by incubation of cultured astrocytes with timosal dramatically increased the amplitude of ATP-induced \([Ca^{2+}]_i\) transients (347). Furthermore, an ATP-induced \([Ca^{2+}]_i\) increase was inhibited by heparin, an intracellular antagonist of InsP_3-gated \(Ca^{2+}\) channels, and prevented by pharmacological inhibition of PLC (375).

The existence of functional P_2 receptors in astroglial cells was demonstrated in situ in Bergmann glial cells studied in acutely isolated cerebellar slices (Fig. 6). Adenosine 5'-triphosphate and P_2a agonists promptly raised \([Ca^{2+}]_i\) (235). As shown in Figure 6, these \(Ca^{2+}\) responses were not affected by removal of external \(Ca^{2+}\) and were not associated with measurable transmembrane currents. Thus intracellular stores appear to be the exclusive source of \(Ca^{2+}\). This was confirmed in experiments in which the ATP-induced \([Ca^{2+}]_i\) rise was blocked by blockade of ER pumps with thapsigargin, as well as intracellular perfusion of these cells with heparin. Such experiments confirm that astrocyte-induced \(Ca^{2+}\) release from ER \(Ca^{2+}\) stores is involved in the ATP response (235).

3. Oligodendrocytes

Cells of the oligodendrocyte lineage respond to ATP by generating \([Ca^{2+}]_i\) transients mediated by activation of metabotropic P_2 and/or P_2a purinoreceptors (213, 237, 407). The ATP-driven \([Ca^{2+}]_i\) responses remain in \(Ca^{2+}\)-free solutions and are inhibited by thapsigargin and intracellular dialysis with heparin (237), indicating a role for the InsP_3-mediated intracellular signal transduction chain. Experiments in situ, in corpus callosum slices, also demonstrated ATP-triggered \([Ca^{2+}]_i\) transients in oligodendrocytes (defined by their morphology and ionic current pattern) (237). The ATP-induced \(Ca^{2+}\) signaling in oligodendrocytes in brain slices was not accompanied by any changes in membrane permeability, further supporting the suggestion of an intracellular origin.
FIG. 6. ATP-induced Ca\(^{2+}\) signaling in Bergmann glial cells results exclusively from inositol 1,4,5-trisphosphate (InsP\(_3\))-mediated Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) pools. A: ATP-induced [Ca\(^{2+}\)]\(_i\) transients were measured from “bulk-loaded” Bergmann glial cells (240) by incubating cerebellar slices in fura 2 acetoxymethyl ester (AM)-containing solutions. Addition of ATP triggered an increase in [Ca\(^{2+}\)]\(_i\), that persisted in Ca\(^{2+}\)-free extracellular solution. B: in a similar experiment, incubation of slice in 500 nM thapsigargin completely and irreversibly blocked ATP-induced [Ca\(^{2+}\)]\(_i\) increase. C: addition of heparin via intracellular dialysis with a patch pipette inhibited [Ca\(^{2+}\)]\(_i\) increase induced by ATP. Control [Ca\(^{2+}\)]\(_i\) transient was recorded from fura 2-AM-loaded cells before starting intracellular dialysis. D: illustration of spatial distribution of [Ca\(^{2+}\)]\(_i\), at time of maximum ATP response. Note higher levels of [Ca\(^{2+}\)]\(_i\), in Bergmann glial cell processes as compared with cell body. [From Kirischuk et al. (235).]

4. Microglia

Microglia respond to ATP with complex [Ca\(^{2+}\)]\(_i\) transients comprised of components associated with intracellular Ca\(^{2+}\) release and transmembrane Ca\(^{2+}\) entry (124, 293, 439). The precise identity of the metabotropic P\(_2\) receptor subtype in microglia is not known. Adenosine 5'-triphosphate-driven [Ca\(^{2+}\)]\(_i\) increases in microglial cells could result either from Ca\(^{2+}\) influx via ionotropic purinoceptors or by activation of metabotropic purinoreceptors with subsequent InsP\(_3\)-induced Ca\(^{2+}\) release from internal pools and (possibly) activation of capacitative Ca\(^{2+}\) entry (293). The ionotropic P\(_2\) receptors have been electrophysiologically characterized in experiments on both cultured microglial cells and in situ preparations and are believed to be of the P\(_{2\alpha}\) (439) and P\(_{2\beta}\) (160) type. The activation of P\(_{2\alpha}\) receptors reportedly was partially responsible for [Ca\(^{2+}\)]\(_i\) rises in ATP-treated microglial cell lines and in freshly isolated microglial cells (124). The role of metabotropic purinoceptors and the existence of capacitative Ca\(^{2+}\) entry mechanisms in microglia needs further investigation.

C. Monoamines

Monoamines (epinephrine as an adrenal medullary hormone and norepinephrine as a neurotransmitter in both peripheral and CNS) exert their physiological action via a broad family of adrenoreceptors (AR). There are three basic subtypes of adrenoreceptors (\(\alpha_1\)-AR, \(\alpha_2\)-AR, and \(\beta\)-AR) that are coupled to different signal transduction systems and have distinct pharmacological profiles (56). The \(\alpha_1\)-AR are coupled via G proteins with PLC and/or plasmalemmal Ca\(^{2+}\) channels and produce an increase in [Ca\(^{2+}\)]\(_i\), in many tissues, whereas \(\alpha_2\)-AR and \(\beta\)-AR con-
trol the activity of adenylate cyclase. In glial cells, the expression of adrenoreceptors and monoamine-triggered Ca\(^{2+}\) signaling has been found exclusively in astrocytes.

Astrocytes express \(\beta\)-AR (184, 276) and \(\alpha_1\)-AR (256, 383) both in culture and in situ (see also Ref. 284 for review of AR in astrocytes). In cultured astrocytes, the expression of \(\beta\)-AR was only found in type 1-like cells (51); \(\alpha_1\)-AR are abundant in all types of astrocytes. Stimulation of \(\alpha_1\)-AR with norepinephrine increases intracellular levels of InsP\(_3\) (337, 339). Microfluorometric [Ca\(^{2+}\)]\(_i\) recording techniques revealed that norepinephrine and other monoamines increased cytoplasmic Ca\(^{2+}\) in cultured astrocytes (49, 193, 285, 317, 373). The pharmacological profile of the monoamine-induced [Ca\(^{2+}\)]\(_i\), transients demonstrated a leading role for the \(\alpha_1\)-AR receptor, although several reports point out that activation of \(\alpha_2\)-AR can increase [Ca\(^{2+}\)]\(_i\), (285, 315, 461), presumably involving PLC-driven production of InsP\(_3\) (as revealed by the inhibition of \(\alpha_2\)-AR-stimulated [Ca\(^{2+}\)]\(_i\), elevation with the specific PLC blocker U-73122; Ref. 114). The density of \(\alpha_1\)-AR linked to the generation of cytoplasmic Ca\(^{2+}\) signals was significantly upregulated by incubation of cortical astrocytic cultures with dibutyryl cAMP (114). The \(\alpha_1\)-AR-mediated [Ca\(^{2+}\)]\(_i\), transients are characterized by complex kinetics, often consisting of an initial peak followed by a long-lasting plateau or oscillations (e.g., Refs. 373, 450). The norepinephrine-induced [Ca\(^{2+}\)]\(_i\), increase in cultured mouse astrocytes was substantially inhibited by chronic treatment (7–14 days) with 1 mM lithium (63). The latter is known to interfere with InsP\(_3\) signaling pathway and has therapeutic potential as a mood-stabilizing drug. Thus it is not excluded that the psychological effects of lithium are due to modulating astrocytic rather than neuronal receptor.

Shao and McCarthy (379, 382) determined the relation between the amplitude of the [Ca\(^{2+}\)]\(_i\), increase in cortical astrocytes and agonist concentration (379, 382). The threshold phenylephrine (PE) concentration was \(~1\) \(\mu\)M, but PE concentrations exceeding 5 \(\mu\)M triggered a maximal response. Moreover, using subsequent monitoring of \(\alpha_1\)-AR-mediated [Ca\(^{2+}\)]\(_i\), increase and the \(\alpha_1\)-AR receptors density in the same cell, they (379) found that the amplitude of [Ca\(^{2+}\)]\(_i\), response was independent of the density of \(\alpha_1\)-AR (despite that the latter varied between 10 and 2,000 binding sites/1,000 \(\mu\)m\(^2\) membrane area). They suggested that \(\alpha_1\)-AR-mediated InsP\(_3\), formation triggers all-or-nothing Ca\(^{2+}\) release from internal stores. The underlying mechanism is unclear but may involve facilitation of InsP\(_3\)-induced release by increased [Ca\(^{2+}\)]. In contrast, experiments on cerebellar Bergmann glial cells (239) revealed a flatter dependence of [Ca\(^{2+}\)], increase on agonist concentration, suggesting a gradual InsP\(_3\)-triggered Ca\(^{2+}\) release in these cells.

Recently, the expression of functional \(\alpha_1\)-AR linked to [Ca\(^{2+}\)], was found in situ in astrocytes from acutely prepared hippocampal stratum radiatum slices (104) and in Bergmann glial cells studied in isolated cerebellar slices (239). In both studies, norepinephrine- and epinephrine-evoked [Ca\(^{2+}\)], responses exhibited \(\alpha_1\)-AR pharmacology (the effects were mimicked by \(\alpha_1\)-AR agonist PE, blocked by \(\alpha_1\)-AR antagonist prazozin, and insensitive to \(\alpha_2\)-AR-specific agents). The \(\alpha_1\)-AR-mediated [Ca\(^{2+}\)], responses were characterized by a long-lasting plateau phase that followed the initial [Ca\(^{2+}\)], rise. Removal of [Ca\(^{2+}\)]\(_o\) eliminated the plateau phase in Bergmann glia but not in hippocampal astrocytes.

### D. \(\gamma\)-Aminobutyric Acid and Glycine

\(\gamma\)-Aminobutyric acid (GABA) effects in the nervous system are mediated by three distinct subtypes of receptors: GABA\(_A\), and GABA\(_C\), are intrinsic ligand-gated Cl\(^-\) channels, whereas GABA\(_B\) receptors are coupled to their effectors (K\(^+\)- or Ca\(^{2+}\)-permeable plasmalemmal channels) via G proteins (206, 308, 386). In contrast to neurons, where GABA normally leads to a hyperpolarization, in macroglial cells GABA depolarizes the membrane (132, 221, 222, 435). The difference is not due to the receptor but rather due to the different intracellular Cl\(^-\) levels in the two cell types. Gial cells have a more positive Cl\(^-\) equilibrium potential \((E_{Cl}\)) than neurons due to a higher intracellular Cl\(^-\) concentration. This is primarily due to the activity of two inwardly directed Cl\(^-\) transporters, a Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter and a Cl\(^-\)/HCO\(_3\)\(^-\) exchanger. The Cl\(^-\) reversal potential in glial cells is about –35 mV, and opening Cl\(^-\) channels led to a depolarizing Cl\(^-\) efflux (132, 435). The GABA-activated inward currents were recorded in cells from astrocyte and oligodendrocyte lineage employing both single cell (45, 131, 221) and brain slice models (32, 398).

In many cases, GABA-induced depolarization of glial cells exceeds the threshold for voltage-gated Ca\(^{2+}\) channels and thereby produces Ca\(^{2+}\) influx and measurable [Ca\(^{2+}\)], transients. Such effects have been recorded from cultured (314) and freshly isolated (131) astrocytes as well as cultured cells of the oligodendrocyte lineage (230). Experiments with freshly isolated astrocytes and oligodendrocytes in culture demonstrated that GABA-induced [Ca\(^{2+}\)], increases were exclusively due to Ca\(^{2+}\) entry via voltage-gated channels, since removal of [Ca\(^{2+}\)], or Ca\(^{2+}\)-channel blockers inhibited the [Ca\(^{2+}\)], rise (131, 230). In experiments on cultured astrocytes, Nilson et al. (314) suggested that in addition to plasmalemmal Ca\(^{2+}\) entry, GABA triggered a release of Ca\(^{2+}\) from intracellular stores, suggesting a possible involvement of GABA\(_B\) receptors. Another piece of evidence suggesting the expression of GABA\(_B\) receptors coupled to Ca\(^{2+}\) entry pathway came from \(^{45}\)Ca\(^{2+}\) flux measurements (3), although this observation has not been substantiated yet with physiological techniques.
Some glial cells have been reported to express glycine receptors that also increase Cl− permeability (231). Activation of these receptors causes depolarization of oligodendrocytes and might also increase [Ca2+], but this has not yet been shown.

E. Acetylcholine

Acetylcholine reacts with two distinct families of cholinoreceptors, nicotinic cholinoreceptors (NChRs) that contain an integral cationic channel and metabotropic muscarinic cholinoreceptors (MChRs) that are coupled to PLC and adenylyl cyclase by means of G proteins (58, 122). The NChRs are assembled from a broad family of elementary subunits belonging to a distinct gene family; some NChR heteromers have relatively high Ca2+ permeability (408, 433). There are five major subtypes of MChRs (M1–M5) that have been identified and cloned (41). They have the seven membrane spanning domain structure typical for metabotropic receptors. Type 1, 3, and 5 MChRs are coupled with PLC, thereby controlling InsP3 turnover, and MChRs types 2 and 4 decrease adenylyl cyclase activity.

In glial cells, NChRs have only been described in invertebrates. Stimulation of NChRs in leech neuropile glial cells causes depolarization (18, 376) and increased [Ca2+], due to both Ca2+ entry via NChRs and voltage-activated plasmalemmal channels (376). In insects, there is evidence of NChRs from immunocytochemical studies (255).

Muscarinic receptors are widely distributed in mammalian macroglial cells. Initially, MChRs were detected in both astrocytes (185, 366) and oligodendrocytes (366) by a variety of histochemical and immunocytochemical methods. Stimulation of MChRs leads to accumulation of InsP3 in glial cell lines (250, 306) and macroglial cells (astrocytes, Refs. 302, 337, 339; oligodendrocytes, Refs. 78, 366). Thus InsP3-mediated [Ca2+], increases were recorded from many types of glial cells treated with MChR agonists. This included astrocytoma cells (286, 320, 327), perisynaptic Schwann cells at the frog neuromuscular junction (200), primary cultured astrocytes (92, 382), and cultured oligodendrocytes (78, 213). In the latter, PCR was used to demonstrate the expression of both M1 and M2 MChRs. However, the carbachol-induced [Ca2+], increase was preferentially sensitive to the M1 agonist pirenzepine, rather than the M2 antagonist methoctramin. This suggests that InsP3 formation and Ca2+ mobilization in oligodendrocytes involves M1 muscarinic receptors (78). Interestingly, MChR-mediated [Ca2+], responses in cells of the oligodendrocyte lineage have two components, an initial rise associated with intracellular Ca2+ release and a long-lasting plateau resulting from Ca2+ entry (78) via an unknown mechanism. Both Ca2+ release and Ca2+ entry are important for stimulation of c-fos expression, which followed the activation of muscarinic receptors in oligodendrocyte precursors (79).

Pharmacological assays indicate that astrocytes also have both M1 and M2 receptors (8, 302) and that M1 receptors are primarily responsible for InsP3 production (8). In addition, in astrocytes from neonatal rat cerebral cortex, M5 receptors coupled to phosphoinositide hydrolysis were found (243). The expression of functional MChRs receptors varies considerably between astrocytes isolated from different brain regions; the carbachol-stimulated InsP3 production mediated by M1 receptors was much higher in astrocytes derived from mesencephalon than in cells from the medulla-pons and cerebral hemispheres, being another example of functional heterogeneity among astrocytes (8).

F. Histamine

Histamine affects three types of receptors: H1 (coupled with PLC), H2 (positively connected with adenylyl cyclase), and H3 receptors that control histamine turnover and release (257). Histaminergic nerve terminals in the posterior hypothalamus release the transmitter nonsynthetically directly into the interstitial space (196). It is thus likely that adjacent glial cells equipped with the proper receptor are activated. Indeed, astrocytes as well as astrocytoma cell lines have both H1 and H2 receptors (187, 195, 196), and stimulation of H1 receptors increased intracellular InsP3 in these cells (10, 244, 305). Measurements of [Ca2+]i revealed intracellular Ca2+ transients in both astrocytoma cell lines (286, 456) and primary cultured astrocytes (136, 193, 194, 285) mediated via H1 histamine receptors. Experiments with cultured cortical astrocytes indicated that H1 receptors and histamine-evoked [Ca2+]i responses were mainly confined to type 2 astrocytes and a subpopulation of type 1 astrocytes (136, 244). The expression of H1 receptors coupled to [Ca2+]i, was recently confirmed in experiments in situ in cerebellar Bergmann glial cells (239). In these cells, histamine induced [Ca2+]i transients in a dose-dependent manner (threshold ~ 1 μM; and maximal response at ~ 100 μM). The histamine-induced [Ca2+]i transients in Bergmann glial cells were sensitive to the H3 specific antagonist chlorpheniramine but were not mimicked by H2 and H3 agonists (dimarit and α-methylhistamine, respectively). Similarly, H2 and H3 antagonists (ranitidine and thioperamide) had no effect. Pre-treatment of Bergmann glial cells with thapsigargin abolished the histamine-induced [Ca2+]i response, suggesting that intracellular Ca2+ release was responsible for the H1 receptor-sensitive [Ca2+]i rise.

In cells of the oligodendrocyte lineage, histamine increased [Ca2+]i in a subpopulation of cultured progenitors and mature oligodendrocytes (213).
G. Substance P

Substance P belongs to a family of neuropeptides, the neurokinins, that affects neurons by stimulating neurokinin receptors widely distributed in the brain (275). These receptors are represented by three subtypes (NK₁, NK₂, NK₃) identified by different molecular structures and a distinct pharmacological profile (89, 384, 458). Among them, NK₁ receptors preferably bind substance P. All three NK receptors are typical G protein-coupled metabotropic receptors with a seven transmembrane domain structure. Interaction of substance P with NK₁ receptors results in phosphoinositide hydrolysis (278, 279) in oligodendrocytes and type 2 astrocytes. Substance P and NK₁ agonists increase [Ca²⁺], by releasing Ca²⁺ from thapsigargin-sensitive stores in astrocytoma cell lines (43, 110, 353) in human glioma line (173), in primary cultured astrocytes (193, 278, 281), and in cultured cells of oligodendrocyte lineage (172, 213). In astrogia, substance P-dependent InsP₃ production and subsequent intracellular Ca²⁺ release was restricted to type 2 astrocytes (278). The NK₁-mediated [Ca²⁺] responses, found in experiments with both glial cells lines (43) and primary cultured oligodendrocytes (172), were relatively short and not affected by extracellular Ca²⁺ removal. This suggests that the Ca²⁺ is released from intracellular stores. Substance P-induced Ca²⁺ signals stimulate interleukin-1 production in cultured astrocytes (281). The activation of NK₁ receptors is accompanied by astrocyte depolarization (444) due to the closure of K⁺ channels (15). What is the mechanism of K⁺ channels inhibition upon substance P stimulation remains unclear; it could be mediated by an [Ca²⁺] increase. This depolarization, in turn, could activate additional Ca²⁺ influx through voltage-gated channels.

H. Bradykinin

Two types of bradykinin (BK) receptors (B₁ and B₂) have been described (161). Both subtypes belong to the G protein-coupled metabotropic receptors linked to PLC and InsP₃ production. Bradykinin acts not only as an effective vasodilator but also as a neurotransmitter. Bradykinin and its binding sites were found in the mammalian brain (135, 344), and various neurons express functional BK receptors. Initial evidence for the presence of BK receptors in glial cells came from biochemical experiments that identified BK binding sites on cultured astrocytes (68) and demonstrated stimulation of phosphoinositide turnover in BK-treated cultured astrocytes (69, 278, 366) and oligodendrocytes (278, 400). In physiological experiments, using microfluorometric [Ca²⁺] recordings, BK was found to increase [Ca²⁺] in cultured Schwann cells (330) and in cultured astrocytes (152, 399) and oligodendrocytes (213, 278). However, in freshly isolated Schwann cells, a BK-induced [Ca²⁺], rise was observed only in a minor cell population (267). The BK-triggered [Ca²⁺] elevation in cultured astroglia was mediated mainly by B₂ receptors as judged by its pharmacological profile, although a minor subpopulation of astrocytes may also contain B₁ receptors (152, 399). The relative importance of intracellular Ca²⁺ release/Ca²⁺ entry components in BK-induced [Ca²⁺], elevation remains unclear. In one series of experiments on cultured astrocytes, BK-triggered [Ca²⁺], transients consist of an initial Ca²⁺ release from ER stores followed by long-lasting Ca²⁺ entry component (152). Another study with the same type of cells found only the Ca²⁺ release component (399). Apart from an [Ca²⁺], rise, an inward current was observed in cultured astrocytes upon BK application (152). The BK-dependent membrane current was also sensitive to B₂ receptor antagonists. However, a clear disparity in the concentration dependence between changes in [Ca²⁺], and membrane conductance was found. The 50% effective concentration (EC₅₀) for [Ca²⁺], rise was ~10 nM BK, whereas EC₅₀ for BK-induced currents was ~1 μM. These results suggest that distinct concentration-dependent signaling pathways aimed at different targets are triggered by activation of B₂ receptors on cultured astrocytes. Bradykinin-induced Ca²⁺ signaling was observed in ~50–60% of cultured astrocytes and was a subject of developmental regulation in oligodendroglia, i.e., BK triggered [Ca²⁺], elevation in ~50% of mature oligodendrocytes compared with only 13% of oligodendrocyte precursors (213).

I. Endothelins

Endothelins, represented by three major isofoms encoded by distinct genes (ET-1, -2, -3; Ref. 197), were first found to be potent vasoactive compounds (455). Later, expression of both endothelin mRNAs and a peptide proper, as well as endothelin binding sites, were found to be widely distributed in the brain (148, 208), and physiological responses were detected in neurons challenged with endothelins (70, 299). Endothelin-induced cellular reactions are mediated via three major receptor subtypes (ETRs) bearing different sensitivity to endothelin isoforms. The most abundant form in the brain is the nonselective ET₃R (which is equally sensitive to all 3 endothelins). The ET₁R (preferably sensitive to ET-1 and ET-2, but not ET-3) is much less expressed. The ET₂Rs have been described only in nonmammalian tissues (26, 372). All endothelin receptors have an overall topology similar to G protein-coupled seven transmembrane domain metabotropic receptors, and their activation involves various G proteins (either PTX sensitive or insensitive). The ETRs are significantly involved in regulation in [Ca²⁺]. They trigger the PLC-InsP₃-Ca²⁺ release transduction chain as well as stimulate transmembrane Ca²⁺ influx (372).

Initially, endothelin receptors were found with autoradiography in various subpopulations of brain astrocytes.
(186). Later, astrocytes were shown to express ET<sub>A</sub> and ET<sub>B</sub> receptors (111, 123, 162). Furthermore, in certain brain regions, expression of endothelin-binding sites was found to be glial specific. The endothelin binding in the cerebellum was quite similar in normal mice and in mutants lacking Purkinje neurons (p<sub>cd</sub> mutants), suggesting that glial cells carry the majority of ETRs (270). Stimulation with endothelin led to an increase of InsP<sub>3</sub> in astrocytes and astroglia cells (70, 86, 263, 270, 459) and to increases in [Ca<sup>2+</sup>]<sub>i</sub>, in both glial cell lines (263, 456, 459) and cultured astrocytes (155, 183, 280, 396, 404); recently, ET-1-induced [Ca<sup>2+</sup>]<sub>i</sub> transients were also detected in microglial cells (293). The glial [Ca<sup>2+</sup>]<sub>i</sub> response to endothelins was found to be insensitive to PTX (183, 396). The endothelin-mediated Ca<sup>2+</sup> mobilization in glialoma cells was inhibited by opioid agonists presumably because they inhibit InsP<sub>3</sub> production (20). The expression of ETRs linked to [Ca<sup>2+</sup>]<sub>i</sub> increases appears to be region specific, i.e., in cortical type 1 astrocytes, endothelin effects on [Ca<sup>2+</sup>]<sub>i</sub> were mediated via ET<sub>A</sub>R (183), whereas cerebellar astrocytes predominantly expressed ET<sub>B</sub>R (396). The astrocytic expression of ETRs linked to [Ca<sup>2+</sup>]<sub>i</sub> was recently demonstrated in experiments in situ on Bergmann glial cells, in which endothelin produced an elevation in [Ca<sup>2+</sup>]<sub>i</sub> (155, 183). The Ca<sup>2+</sup> responses in Bergmann glial cells were mediated by ET<sub>B</sub>R (418) as was revealed by single-cell RT-PCR analysis.

The endothelin-induced [Ca<sup>2+</sup>]<sub>i</sub> transients in both cultured and in situ astrocytes had heterogeneous kinetic properties varying from a simple peak response to a bi-phasic [Ca<sup>2+</sup>]<sub>i</sub> rise, with a plateau lasting up to 20 min after agonist washout (155, 183). Sometimes endothelin triggered only a plateau without a defined peak (183). As in the case of other agonist-triggered [Ca<sup>2+</sup>]<sub>i</sub> responses, the initial peak resulted from internal Ca<sup>2+</sup><sup>2+</sup> release, while the plateau phase was entirely dependent on extracellular Ca<sup>2+</sup> (183, 263, 396, 404, 419). The nature of Ca<sup>2+</sup> delivery pathway underlying the plateau phase of endothelin-induced responses remains controversial. Studying endothelin responses in cerebellar cultured glial cells, one study found it was completely (404) or partially (396) inhibited by nifedipine, suggesting an important role for voltage-gated Ca<sup>2+</sup> channels, whereas in another study, nifedipine had only a marginal effect (183). The effects of ETR stimulation on Ca<sup>2+</sup> channels might be mediated by diacylglycerol, since treating cultured astrocytes with a protein kinase inhibitor causes a nifedipine-sensitive plateau (183). Diacylglycerol might induce phosphorylation of Ca<sup>2+</sup> channels and shift their threshold toward more negative membrane potentials. The participation of Ca<sup>2+</sup> channels in the response may vary considerably from cell to cell. A second possible mechanism for producing a prominent plateau phase in endothelin-induced [Ca<sup>2+</sup>]<sub>i</sub> transients might be either endothelin-activated plasmaemmal channels or capacitative Ca<sup>2+</sup> entry. The capacitative Ca<sup>2+</sup> entry pathway is likely to shape the plateau of endothelin-induced Ca<sup>2+</sup> responses in Bergmann glial cells in situ (418). In the same cells, endothelin-triggered [Ca<sup>2+</sup>]<sub>i</sub> transients exhibited a progressive rundown with repeated endothelin applications (418). This could be the result of the internalization of the ETRs (364).

Endothelin-induced [Ca<sup>2+</sup>]<sub>i</sub> signaling might participate in the astroglial proliferative response. Increased levels of endothelins in brain tissue accompany various insults in the CNS (22, 153). This observation suggests the hypothesis that ETRs are involved in the transduction of a mitogenic signal which, at least partially, underlies the activation of astrocytes involved in brain pathology. Ischemic conditions are also accompanied by an increase in endothelin synthesis (22), and endothelin antagonists are neuroprotective during brain ischemia (125). It has been demonstrated that endothelin-induced [Ca<sup>2+</sup>]<sub>i</sub> increases play a pivotal role in stimulation of DNA synthesis and proliferative response of cultured type 1 astrocytes (396, 404). In addition, endothelins were found to trigger immediate early genes expression; the level of Fox proteins increases significantly in endothelin-challenged glial cells from organotypically cultured cerebellar slices (403). This suggests that ET receptors can play a role in development or during pathological events when glial cells undergo phases of proliferation. In this context, the glial endothelin system has been implicated in a number of pathophysiological situations such as viral infections (269), Alzheimer’s disease (460), and ischemia (454).

J. Other Agonists Linked to Intracellular Ca<sup>2+</sup> Regulation in Glial Cells

1. Serotonin

Serotonin is a neurotransmitter in a number of brain regions. It exerts its action via a broad spectrum of receptors that have been classified into four subfamilies (5-HT<sub>1</sub> to 5-HT<sub>4</sub>). Both 5-HT<sub>1</sub> and 5-HT<sub>4</sub> are coupled to adenylate cyclase, whereas 5-HT<sub>2</sub> receptors are ligand-operated cationic channels and 5-HT<sub>2</sub> receptors are positively coupled with PLC, thereby regulating InsP<sub>3</sub> production (189, 462). Serotonin activates phosphoinositide turnover in glial cell lines (7) and raises [Ca<sup>2+</sup>]<sub>i</sub>, in astrocytotmas (325, 402) and subpopulations of primary cultured astrocytes (94, 193, 316, 425). Type 1 astrocytes are more sensitive to serotonin (285) than type 2 astrocytes (92). Cultured astrocytes challenged with serotonin display kinetically different responses varying from single spikes to prolonged oscillations (316). In Schwann cells, results on the appearance of 5-HT receptors linked to [Ca<sup>2+</sup>]<sub>i</sub> are controversial. In a comprehensive study of neuroactive substances, Lyons et al. (267) failed to detect an effect of serotonin on [Ca<sup>2+</sup>]<sub>i</sub>, in cultured Schwann cells. Another study of cultured Schwann cells (457) found a consistent increase in [Ca<sup>2+</sup>]<sub>i</sub>, mediated by stimulation 5-HT<sub>2A</sub> receptors. In the latter
case, the Schwann cells were maintained in 4 μM forskolin; such treatment could promote the expression of serotonin receptors. In cultured astrocytes, serotonin effects were mediated by 5-HT2 receptors, as detected by radioligand binding, Northern blots, and pharmacological assays (94). Further studies revealed the pharmacological profile of these receptors corresponds to the 5-HT2c subtype (64).

2. Oxytocin and vasopressin

Receptors for the closely related neurohormones oxytocin and vasopressin have been found in several types of astroglial cells. Oxytocin caused an increase in InsP3 level in cultured astrocytes from cortex and cerebellum but not from spinal cord (67). The oxytocin binding sites were initially described in cultured hypothalamic astrocytes (100, 101), and later, functional oxytocin receptors linked to intracellular Ca2+ signaling were found in the same cells (99). Oxytocin-triggered [Ca2+]i transients were observed in a majority of hypothalamic astrocytes. The responses usually had a monophasic peak with a fast decay to the initial [Ca2+]i level. A minority of cells (15%) underwent [Ca2+]i oscillations. The transients remained in Ca2+-deficient extracellular media, suggesting they originated from intracellular Ca2+ release. They exhibited a prominent rundown in response to successive oxytocin applications apparently due to receptor desensitization rather than depletion of stores (99).

The addition of vasopressin to cultured cortical and cerebellar astrocytes also increased intracellular InsP3 (67). Subpopulations of cultured type 2 astrocytes (193), astrocytes derived from rat neural lobes (168) and circumventricular organs (210), also responded to vasopressin with an increase in [Ca2+]i. Naturally occurring fragments of vasopressin increased [Ca2+]i in cultured astrocytes from the circumventricular organ (211). The effects of vasopressin on [Ca2+]i, in astrocytes were mediated by V1 vasopressin receptors, as suggested by their pharmacological profile (210, 211). Vasopressin-triggered [Ca2+]i transients were maintained in Ca2+-free solutions, indicating that they resulted (as in the case of oxytocin) from a release of Ca2+ from intracellular stores (210).

3. Neuropeptide Y

Neuropeptide Y (NPY), a member of the pancreatic polypeptide family (which includes also pancreatic polypeptide and peptide YY), was found to be expressed in the CNS where it exerts various regulatory effects (see Refs. 80, 106, 443 for review). In astrocytes, the specific binding of 125I-NPY was detected, suggesting the existence of specific receptors (151). Applications of NPY, in micromolar concentrations, to primary cultured cortical astrocytes caused an increase in [Ca2+]i, (151). This increase was observed in 20–70% of cells in different preparations and appeared to result entirely from Ca2+ influx via voltage-gated channels, since either removal of Ca2+ from the bath or the addition of Cd2+ (2 mM) prevented the [Ca2+]i responses to NPY. The channels were activated by NPY-triggered cell depolarization resulting from an inward current in response to NPY application. This current resulted from the closing of K+ channels. The NPY-dependent modulation of K+ channels was PTX insensitive (151).

4. Complement fragments

Complement fragments (anaphylatoxins) are generated during the activation of the complement system and play an important role in various immunological reactions (157). Recently, it was discovered that complement fragments can be directly produced in the brain (21). The action of anaphylatoxins is achieved via activation of specific receptors belonging to the metabotropic family and have a characteristic seven transmembrane structure (363, 365). They are believed to regulate PLC-controlled InsP3 accumulation and initiate Ca2+ release from intracellular stores (294, 319). Initially, expression of complement fragments receptors was thought to be a perogative of leukocytes. However, functional anaphylatoxin receptors were found in other cells including neuroglia. The receptors for complement fragment C5a were found in both primary cultured human fetal astrocytes and in a human astrocyte cell line. Stimulation of these receptors raised intracellular [Ca2+]i in cultured astrocytes (144). Similarly, complement fragments C5a and C3a triggered a transient [Ca2+]i rise in both cultured microglial cells and microglial cells on the surface of acutely isolated corpus callosum slices (292). The anaphylatoxin-induced [Ca2+]i transients recorded in microglial cells result from Ca2+ release from intracellular stores followed by a capacitative Ca2+ influx (292).

5. Platelet-activating factor

Platelet-activating factor (PAF) is known to substantially modulate neuronal functions by exerting a pleiotropic effect and modulating synaptic transmission (27, 215). Its effect is mediated via stimulation of specific PAF receptors (PAFR; Ref. 38), which control phosphoinositide turnover and induce Ca2+ release from internal stores in various types of cells, including neurons (39, 445). The PAFRs have been also found in C6 glioma cells, primary cultured astrocytes, and oligodendrocytes (48). In cultured astrocytes, PAF was reported to increase cytoplasmic InsP3 (346). High expression of PAFR and PAF-induced [Ca2+]i elevation (due to both internal Ca2+ release and Ca2+ influx) has also been detected in immortalized astrocytes and cultured microglial cells (293, 298) microglial cells.

6. Prostanoids

Prostanoid receptors constitute a large family (442); these receptors are coupled to cAMP turnover (DP, EP2,
and IP receptors) and to PLC and InsP3 production (EP1, EP3, FP and TP receptors). The expression of FP and TP receptors linked to phosphoinositide hydrolysis was demonstrated in human astrocytoma cell line (241), and FP receptor-mediated Ca2+ increases were found in primary cultured type 1 astrocytes (198).

7. Vasoactive intestinal polypeptide

Vasoactive intestinal polypeptide (VIP) in concentrations of 0.1–1 mM was found to increase [Ca2+]i in a subpopulation of cultured cortical type 1 astrocytes (119). Simultaneous administration of VIP and subthreshold concentrations of norepinephrine greatly increased the percentage of responding astrocytes and enhanced the VIP [Ca2+]i response. The VIP-triggered [Ca2+]i increases persist in Ca2+-free external solutions and are blocked by thapsigargin, suggesting they result from intracellular Ca2+ release. Another endogenous ligand for VIP receptors, pituitary adenylate cyclase-activating polypeptide, releases Ca2+ from internal stores in type 2 but not in type 1 astrocytes (415).

8. Platelet-derived growth factor

Examples of platelet-derived growth factors (PDGFs) are three proteins that are potent mitogenes (174). The PDGF receptors (α and β) have been found in oligodendrocytes and Schwann cells, and PDGF was shown to promote proliferation of various types of glial cells (360). Intracellular Ca2+ transients as well as [Ca2+]i oscillations have been found in oligodendrocyte cell line (120) and in oligodendrocyte precursors (165) in response to PDGF. In the latter, PDGF-induced Ca2+ signaling may be involved in the initiation of cell differentiation.

9. Angiotensin II

Angiotensin II-induced [Ca2+]i increases have been demonstrated in human astrocytoma cell lines (412) and in several types of primary cultured astrocytes (201, 441). In astroglial cultures derived from the hypothalamus and brain stem of adult rat, angiotensin II triggered [Ca2+]i transients with a clear biphasic (peak followed with plateau) time course. The initial peak resulted from Ca2+ release from the internal stores (as judged by its insensitivity to [Ca2+]i, and inhibition by thapsigargin and cyclopiazonic acid), whereas the plateau component reflected Ca2+ entry via nifedipine and cadmium-sensitive plasmalessunl Ca2+ channels (441). The effects of angiotensin on [Ca2+]i in astrocytes were mediated via AT1 receptors (441).

10. Serum albumin and thrombin

Dialyzed serum was reported to trigger [Ca2+]i increases in primary cultured cerebellar astrocytes (321). Other blood-derived proteins, albumin and thrombin, have been reported to affect [Ca2+]i in several types of cells of glial origin. Albumin was reported to have a rather unusual effect on [Ca2+]i, in cultured astrocytes, decreasing [Ca2+]i at low concentrations (<2 mg/ml) and initiating [Ca2+]i spikes at high concentrations (303, 304). The decrease in [Ca2+]i, in the presence of low albumin concentrations was accompanied by a significant rise in the amplitude of thapsigargin- and glutamate-triggered [Ca2+]i transients. This suggested that albumin promotes Ca2+ accumulation into the ER Ca2+ stores (303). As albumin concentrations rise in the brain after injury, this effect would potentiate neurotransmitter-induced Ca2+ signals in astrocytes by increasing the releasable Ca2+ content in intracellular stores. Such a potentiation might also be relevant for astrocyte responses that are an important defense component during brain insults. Another blood factor, thrombin, was reported to cause [Ca2+]i oscillations in astrocytoma cells in nanomolar concentrations. Similar oscillations were produced by thrombin receptor-activating peptide. This suggests the involvement of a specific plasmenemal thrombin receptor (87).

11. Arachidonic acid

Arachidonic acid, which serves as a biologically active signaling molecule, was reported to directly raise [Ca2+]i in cultured rat spinal cord oligodendrocytes (391). This [Ca2+]i rise appeared to result from stimulation of a Ca2+ influx pathway.

12. Opioids

Several groups have reported that activation of opioid receptors trigger increase in [Ca2+]i, in cultured astrocytes. The precise nature of receptors involved remains unclear. In astrocytes cultures from newborn mice cerebrum (401) and rat cortex (116), morphine produced [Ca2+]i increases via activation of κ-opioid receptors (as [Ca2+]i, responses were mimicked by selective κ-receptor agonist U-69593). In contrast, another group (170) reported that morphine’s effect on [Ca2+]i, in cultured mice brain astrocytes was mediated through μ-opioid receptors. In the latter preparation, morphine-activated [Ca2+]i transients were simulated by μ-receptor agonist PL-017, and furthermore, expression of μ-receptors was substantiated by immunostaining with specific antibodies. The intracellular mechanism for morphine-induced [Ca2+]i mobilization is also unclear. Eriksson et al. (116) demonstrated that stimulation of κ-opioid receptors results in a stimulation of Ca2+ influx through L-type voltage-gated channels. In contrast, Hauser et al. (170) believe that morphine triggers Ca2+ release from thapsigargin- and dantrolene-sensitive intracellular deposits (170). Stimulation of opioid receptors in astrocytes also affects DNA synthesis and astrocytic growth. The role of [Ca2+]i, in mediating these effects re-
mains obscure, although it might be associated with intracellular Ca$^{2+}$ release (170).

13. **Myelin**

It is of interest that myelin, the main product of differentiated oligodendrocytes, produces [Ca$^{2+}$], transients in both oligodendrocytes (297) and brain stem neurons (296) in vitro. This is accompanied by a block of oligodendrocytic motility and collapse of neuronal growth cones. The mechanisms for myelin-triggered Ca$^{2+}$ signaling substantially differed between neurons and oligodendrocytes. In neurons, myelin promoted Ca$^{2+}$ influx via voltage-gated channels, whereas in oligodendrocytes, most of the Ca$^{2+}$ was released from intracellular stores. Thus, with the use of Ca$^{2+}$ signaling, oligodendrocytes could recognize contact with other oligodendrocytes, although the details of the myelin-triggered changes in [Ca$^{2+}$] remain unclear. The myelin basic protein in micromolar concentrations also triggered a prominent [Ca$^{2+}$], increase associated with Ca$^{2+}$ influx (420); this [Ca$^{2+}$] increase was fatal for oligodendrocytes.

14. **Benzodiazepine receptors**

Apart of acting on GABA$_A$ receptors, benzodiazepines exert their action through a not yet precisely characterized set of binding sites found in a variety of mammalian cells and in glial cells in particular (447). Stimulation of cultured rat astrocytes with endogenous benzodiazepine receptor ligand octadecaneuropeptide in nanomolar (0.1±10 nM) concentration triggered [Ca$^{2+}$], transients (251). This Ca$^{2+}$ elevation was independent of extracellular Ca$^{2+}$, blocked by thapsigargin or by incubation with PTX for 4 h. These data obviously suggest that benzodiazepine receptors in astrocytes are coupled with the generation of Ca$^{2+}$ release from, presumably, InsP$_3$-sensitive stores.

K. **Heterogeneity of Neurotransmitter Receptor Expression in Glial Cells**

The data on the presence of neurotransmitter receptors presented above clearly demonstrate that glial cells can express functional receptors to almost all known neurotransmitters, neuromodulators, and neurohormones (Table 2). The important question is whether all these receptors are expressed in situ and which factors regulate their expression. Most of the experiments on glial receptors have been carried out in tissue-culture systems; our knowledge of the receptor pattern expressed by glial cells in vivo is far more limited. Glial cells in culture express a remarkable heterogeneity in their sensitivity to various neuroligands. Cultured astrocytes can respond at the same time to a number of ligands with an increase in [Ca$^{2+}$]. The pattern of receptor expression can differ substantially between cells in the same culture (92, 285, 380, 381). Moreover, the same heterogeneity persists within clones of cultured astrocytes, suggesting that the receptor pattern is not inherited from the parent astrocyte (380). In a series of elegant experiments, Shao and McCarthy (380, 381) demonstrated that the [Ca$^{2+}$], responsiveness to various neuroligands can differ between two sister cells immediately after astrocyte division. Thus astrocytes are not born with a fixed set of functional receptors but can vary the expression of a variety of neurotransmitter receptors depending on factors in their environment.

Similarly, neuroligand [Ca$^{2+}$], responsiveness of cultured oligodendrocytes is heavily controlled by culture conditions. Even more interesting, the expression of neuroligand receptors linked to the Ca$^{2+}$ signaling appeared to be controlled by oligodendrocyte-neuronal contacts (171). Preventing glial-neuronal contacts by transection of neurites in oligodendroglial-DRG cocultures significantly reduced the number of oligodendrocytes sensitive to a variety of neuroligands (ATP, carbachol, and histamine; responsiveness to BK was not affected; Ref. 171). Neuronal control of receptors expression in neighboring glia appeared to be sensitive to tetrodotoxin, suggesting the important role of neuronal activity.

An important question is whether this plasticity is present not only when astrocytes are in culture but also in vivo. Recently, this question has been investigated in studies of neurotransmitter-induced Ca$^{2+}$ signaling in glial cells in situ, in brain slices. Glial cells (both oligodendrocytes and astrocytes) in corpus callosum slices demonstrated prominent developmental changes in their neurotransmitter receptor expression (33). In slices obtained from young (3–7 days old) mice, glial cells were rather promiscuous, responding with [Ca$^{2+}$] increases to ATP, glutamate, histamine, GABA, norepinephrine, serotonin, angiotensin II, BK, and substance P. In contrast, in older (11–18 days old) animals, the expression of receptors was limited to glutamate, ATP, and norepinephrine. Another comprehensive study of Ca$^{2+}$ signaling-linked neurotransmitter expression has been carried out in cerebellar Bergmann glial cells. These cells provide a unique model for studying glial cells in slices because their characteristic morphology makes identification easy. The study found that, contrary to cultured astrocytes, Bergmann glia always express a distinct set of receptors. These include $\alpha_1$-adrenoreceptors, AMPA/kainate GluRs, mGluRs, H$_1$ histamine receptors, P$_{2Y}$ purinoreceptors, and ET$_B$ endothelin receptors (232, 235, 239, 419, 429). Other substances known to induce Ca$^{2+}$ signals in cultured macroglia were ineffective. Interestingly, the Purkinje cell layer, where Bergmann glial cells are located, receives afferents (323) using the following neurotransmitters: glutamate (parallel fibres), norepinephrine and ATP (terminals from locus ceruleus), and histamine (histaminergic terminals from tuberomammillary nucleus of posterior hypothalamus). Thus Bergmann glial cells express receptors that are ap-
FIG. 7. Bergmann glial cell and its neighbor Purkinje neuron bear a similar set of neurotransmitter receptors. See text for discussion and receptor nomenclature. NT, terminals from tuberomammillary nucleus of posterior hypothalamus which carry histamine innervation of cerebellar cortex; LC, terminals from locus ceruleus which utilize norepinephrine and ATP as neurotransmitters; CF and PF, climbing and parallel fibers, respectively, of major neurotransmitter glutamate; BA and ST, basket and stellate cells which deliver \( \gamma \)-aminobutyric acid (GABA) to Purkinje neuron layer. Glu, glutamate; AMPA, \( \alpha \)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; ET, endothelin; mGluR, metabotropic glutamate receptor; Hist, histamine; NA, norepinephrine. Other definitions are as in Fig. 2.

proper to detect transmitters secreted in their vicinity. Furthermore, it appears that Ca\(^{2+}\) signaling-related receptors expressed by Bergmann glia almost exactly match receptors found on the closely apposed Purkinje neurons (Fig. 7; Refs. 234, 429). An exception was ET\(_{BR}\). Although the mRNA for the latter is expressed in Purkinje neurons, as revealed by single-cell RT-PCR, the endothelins failed to trigger an \([\text{Ca}^{2+}]\) increase (418). Another example of similarity of glial and neuronal receptor expression came from the experiments on spinal cord slices. It appears that the spinal cord is the only region found so far where both oligodendrocytes and astrocytes express glycine receptors, which are known to be present on spinal cord neurons (231, 333). It seems fruitful to pursue comparable studies of the similarity of receptors on adjacent glial cells and neurons in other areas of the nervous system to test the hypothesis that the receptors expressed on glial cells enable the detection of transmitter substances released in the same anatomical region by neurons.

VI. SPATIOTEMPORAL ORGANIZATION OF CALCIUM SIGNALS

A. Intracellular Ca\(^{2+}\) Oscillations

After stimulation, the temporal and spatial distribution of the increase in \([\text{Ca}^{2+}]\) in glial cells, especially astrocytes, is remarkably complex. At a given point, the time course of the increase may be a single spike, biphasic with an initial peak followed by a plateau, or oscillations (129, 429). Usually, simple monophasic increases follow activation of voltage-gated Ca\(^{2+}\) channels and/or ionotropic receptors (e.g., Refs. 103, 199), whereas stimulation of metabotropic receptors, releasing intracellular \([\text{Ca}^{2+}]\), leads to complex biphasic/oscillatory \([\text{Ca}^{2+}]\) responses (61, 84, 225, 450). The initial phase of these complex \([\text{Ca}^{2+}]\) signals follows InsP\(_3\) mediated \([\text{Ca}^{2+}]\) release from intracellular stores. The plateau/oscillation phases requires extracellular \([\text{Ca}^{2+}]\) (154, 155, 183, 203, 362, 419). The \([\text{Ca}^{2+}]\) influx pathway activated with metabotropic receptor stimulation is unclear. By analogy with other tissues (35, 75), the most likely candidate is the store-operated \([\text{Ca}^{2+}]\) channel, although the existence of this pathway in glial cells has not been demonstrated.

The appearance of biphasic/oscillatory \([\text{Ca}^{2+}]\) responses depends on agonist concentration; cultured astrocytes stimulated with low doses of metabotropic agonists responded with \([\text{Ca}^{2+}]\) spikes, whereas higher doses led to an additional plateau phase or \([\text{Ca}^{2+}]\) oscillated. Such a dependence was found for glutamate (1, 154), histamine (136), norepinephrine (373), and endothelin (155). For glutamate, the concentration dependence of \([\text{Ca}^{2+}]\) responses was even more complicated; at low concentrations, monophasic \([\text{Ca}^{2+}]\) transients were recorded, moderate concentration triggered \([\text{Ca}^{2+}]\) oscillations, and at
high glutamate concentrations, the initial \([\text{Ca}^{2+}]\), peak was followed by a sustained \([\text{Ca}^{2+}]\), plateau (84).

In addition to a complex temporal pattern, glial \(\text{Ca}^{2+}\) signaling exhibits spatial heterogeneity. In many glial cell populations, \([\text{Ca}^{2+}]\) rises first in processes and then spreads toward the soma [e.g., in ATP or KCl-stimulated oligodendrocytes (237, 238), in histamine-stimulated astrocytes (194), or in ATP-challenged Bergmann glial cells (235); cf. Figs. 4–6]. Such a pattern is expected from considerations of surface-to-volume relations if the release or influx is constant for a given membrane area. However, subsequent analysis suggests the situation is more complex. The localization of \(\text{Ca}^{2+}\) release sites may reflect a higher concentration of \(\text{Ca}^{2+}\) signaling molecules in distal parts of glial cells in regions where they are in more intimate contact with neurons. Cultured astrocytes display more complex spatial \([\text{Ca}^{2+}]\), signals, developing long-lasting \([\text{Ca}^{2+}]\), waves (450, 451). These waves are associated with several intracellular loci, each characterized by its own oscillatory pattern with \([\text{Ca}^{2+}]\), waves propagating between them (450, 451). Several hypotheses have been proposed to explain \([\text{Ca}^{2+}]\), wave initiation and propagation. These include periodic fluctuations of cytoplasmic \(\text{InsP}_3\) level, \([\text{Ca}^{2+}]\),-dependent regulation of \(\text{InsP}_3\)-gated \(\text{Ca}^{2+}\) release channel open probability, and an interplay between \(\text{InsP}_3\)-induced and \(\text{Ca}^{2+}\)-induced \(\text{Ca}^{2+}\) release (see Refs. 6, 36, 259 for review).

In cultured astrocytes obtained from the visual cortex, the glutamate-evoked \([\text{Ca}^{2+}]\), oscillations demonstrated a long-lasting modulation in frequency. The second application of glutamate arriving 1–60 min after the first challenge always triggered \([\text{Ca}^{2+}]\), oscillation with higher frequency (332). These changes of frequency were exclusively confined to glutamate-induced \(\text{Ca}^{2+}\) signals; \([\text{Ca}^{2+}]\), oscillations triggered by other agonists did not show such long-term plasticity.

**B. Intercellular \(\text{Ca}^{2+}\) Waves**

In cultured astrocytes, a stimulus-evoked intracellular \(\text{Ca}^{2+}\) wave can cross cell boundaries and travel within the astrocytic network. The seminal observation was made by Cornell-Bell et al. (83), who found that in the presence of glutamate, \(\text{Ca}^{2+}\) waves propagate through the astrocyte syncytium. The waves followed complex routes, without delays at cell borders, for hundreds of microns at a velocity of \(~15–20\ \mu\text{m}/\text{s}\) (83, 84, 428). The propagation of glutamate-triggered \(\text{Ca}^{2+}\) waves requires extracellular \(\text{Ca}^{2+}\). An alternative way to induce intercellular \(\text{Ca}^{2+}\) waves is with focal mechanical stimulation. These waves have different properties; they have a delay at cell borders and persist in \(\text{Ca}^{2+}\)-free solutions (61). They are, however, dependent on \(\text{Ca}^{2+}\) release from the internal stores; the depletion of stores with thapsigargin blocks wave propagation (60). The mechanism of intercellular propagation also involves gap junctions; the waves are inhibited by octanol, halothane (115, 126), or by an endogenous derivative of arachidonic acid, anandamide (427). In the C6 glioma cell line, intercellular \(\text{Ca}^{2+}\) wave propagation was observed only in cells that were transfected with the gene for the gap junction protein connexin-43 (62). Thus a likely mechanism for \(\text{Ca}^{2+}\) wave propagation involves intercellular (via gap junctions) diffusion of \(\text{InsP}_3\), which generates \(\text{Ca}^{2+}\) release in one cell after another (126, 390); the degree of intercellular coupling would, presumably, determine the wave path. The important role of intracellular \(\text{Ca}^{2+}\) stores and \(\text{InsP}_3\) production is substantiated by the finding that treatment of astrocytic cultures with thapsigargin or with PLC blocker U-73122 completely prevented the spread of intercellular \(\text{Ca}^{2+}\) waves (428). Interestingly, interglial communications via gap junctions may be regulated by various physiological stimuli, e.g., it increases upon glutamate and high K+ treatment (113, 150) or even after action potentials in adjacent axons (277). This susceptibility of interglial gap junctions to external regulation may influence interglial \(\text{Ca}^{2+}\) signaling.

Alternative mechanisms for \(\text{Ca}^{2+}\) wave propagation may involve an extracellular messenger released by stimulated cells. An extracellular pathway for \(\text{Ca}^{2+}\) wave propagation was observed by Hassinger et al. (167). To test the
importance of the extracellular pathway, they created a cell-free lane in confluent astrocytic cultures by mechanically eliminating astrocytes with a glass pipette. As shown in Fig. 8A, electrically induced Ca\textsuperscript{2+} waves appeared to cross cell free lanes narrower than 120 \(\mu m\). Moreover, the velocity of wave propagation through the cell-free lane did not differ significantly from the velocity of the wave spreading via the astrocytic network. The nature of the extracellular messenger was not clarified, although glutamate can be excluded; the wave spreading over cell-free regions was not modified by glutamate receptor antagonists. Whether the extracellular mechanism may work in concert with gap junction propagation or it may take the leading role in certain brain regions remains totally unclear.

The existence of interastrocytic [Ca\textsuperscript{2+}], waves in brain tissue has only recently been demonstrated. A propagating wave was observed by Dani et al. (90) in organotypic hippocampal slice cultures. These waves were initiated by stimulation of mossy fibers, which are believed to utilize glutamate as a neurotransmitter. However, the intercellular Ca\textsuperscript{2+} waves observed in these experiments usually propagated for much shorter distances (2–3 astrocyte diameters) than observed in cultures. This might reflect a higher degree of coupling of cultured astrocytes than astrocytes in situ. More recently, propagating Ca\textsuperscript{2+} waves were recorded in glial cells from acutely isolated rat retina (312). These Ca\textsuperscript{2+} waves were initiated by either local mechanical or electrical stimulation or by focal application of ATP, carbachol, or phenylephrine (Fig. 8, B and C). Interestingly, focal applications of glutamate did not trigger Ca\textsuperscript{2+} waves in retinal glial networks, although incubation of the whole preparation in glutamate potentiated Ca\textsuperscript{2+} waves induced by other stimuli. The propagation velocity of Ca\textsuperscript{2+} wave in retinal preparation was \(\sim 25 \mu m/s\) for all types of stimulation. The retinal Ca\textsuperscript{2+} waves obviously originated from intracellular Ca\textsuperscript{2+} release, being preserved in Ca\textsuperscript{2+}-free solution and blocked by thapsigargin as shown in Figure 8C.

**VII. GLIAL CALCIUM SIGNALING AND NEURON-GLIAL INTERACTIONS**

The coordination of neuronal and glial activity requires that appropriate signals pass from one cell type to the other. The study of these signals is a central theme in neuroglial research (82, 220, 389). These interactions might involve short-term functions such as ion regulation, release of substrates, or transmitter clearance or much longer term processes as might be involved in neuronal guidance during development, myelination, mitosis, and regeneration. The question of how changes in glial [Ca\textsuperscript{2+}], serve to couple neuronal and glial functions is a major question raised by the work that has been reviewed and, unfortunately, largely unanswered. Most of the studies of [Ca\textsuperscript{2+}], in glial cells have been carried out with astrocytes in tissue culture. These cells have membrane receptors for many neurotransmitters, and their response often includes changes in [Ca\textsuperscript{2+}]. It is tempting to assume that when the transmitters are released from the neurons that they provoke responses not only in a postsynaptic cell but also in the closely apposed glial cells. Moreover, neurons release a variety of substances, e.g., amines, peptides, and hormones, which diffuse through the extracellular space bathing other neurons and glial cells. Among the questions that need to be addressed are the following: 1) Which receptors are expressed in glial cells in the intact nervous system? 2) Can a glial cell distinguish between stimulants that all increase [Ca\textsuperscript{2+}]? 3) What is the functional response of the glial cell after it has undergone an increase in [Ca\textsuperscript{2+}]? 4) What other changes within the glial cell result from neuronal activity in addition to an increase in [Ca\textsuperscript{2+}]?

Because glial Ca\textsuperscript{2+} signaling appears to be a ubiquitous consequence of the activation of glial neurotransmitter receptor systems, it is of interest to explore whether neuronal activity leads to [Ca\textsuperscript{2+}], fluctuations in glial cells. Indeed, experiments performed recently in both peripheral and central macroglial cells demonstrate that glia sense nervous activity, and the latter initiates Ca\textsuperscript{2+} signals in glial cells. The peripheral glia, the Schwann cells, generate [Ca\textsuperscript{2+}], transients in response to electrical stimulation of peripheral nerves (200, 258, 363). In periaxonal Schwann cells, the Ca\textsuperscript{2+} signal probably results from depolarization occurring in response to increases in [K\textsuperscript{+}], resulting from nerve activity; this depolarization, in turn, triggers Ca\textsuperscript{2+} entry via voltage-gated channels and subsequent activation of Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release from internal stores (258). In contrast, in perisynaptic Schwann cells, Ca\textsuperscript{2+} signals were mediated by either muscarinic or P\textsubscript{2} purinergic receptors (200, 363) activated by neurotransmitters released from the nerve ending.

Neuronal activity has been found to induce [Ca\textsuperscript{2+}], elevation in glial cells from the leech nervous system (371). At least in part, these glial Ca\textsuperscript{2+} signals were mediated through activation of GluR. Similarly, in brain white matter, electrical stimulation of the optic nerve evoked oscillatory Ca\textsuperscript{2+} responses in periaxonal glial cells (65, 247); the frequency of [Ca\textsuperscript{2+}], oscillations correlated with stimulation frequency. The possible mechanism underlying axon-glial communication in the optic nerve may involve glutamate release from the stimulated nerve due to reversal of Na\textsuperscript{+}/glutamate transporter (65).

In astrocytes of the gray matter, neuronal-induced Ca\textsuperscript{2+} signaling has also been found in both neuronal-glial cocultures and in hippocampal slice preparations. In neuronal-glial cocultures, selective stimulation of neurons with NMDA triggered [Ca\textsuperscript{2+}], spikes and [Ca\textsuperscript{2+}], oscillations in the neighboring astrocytes (91). Using in situ preparation, Dani et al. (90) observed [Ca\textsuperscript{2+}], transients evoked...
by mossy fiber stimulation in astrocytes in hippocampal cultured slices. Similarly, stimulation of Schaffer collaterals triggered $[Ca^{2+}]_i$, elevation in astrocytes located in CA1 stratum radiatum region (351). In hippocampal and visual cortex slices, electrical stimulation of presynaptic afferents triggered $[Ca^{2+}]$, oscillations in astrocytes (331); moreover, the frequency of astrocytic $[Ca^{2+}]$, oscillations increased while increasing the intensity or frequency of afferent stimulation. In all cases, $[Ca^{2+}]$, transients in astrocytes were mediated by glutamate presumably released from neuronal terminals, since astrocytic $Ca^{2+}$ responses were mimicked by (1S,3R)-ACPD (331) and were inhibited by either nonselective ionotropic GluR blocker kinurenic acid (90, 351) or by mGluR blocker $\alpha$-methyl-4-carboxyphenylglycine (351).

In cocultures, it has been found that not only may neurons initiate $Ca^{2+}$ signals in glial cells, but also glial $[Ca^{2+}]$, waves may trigger $Ca^{2+}$ signals in neurons. First, Nedergaard (310) found that $[Ca^{2+}]$, waves in astrocytes in rat forebrain cortical cultures triggered $[Ca^{2+}]$, spikes in neurons. This astrocyte-to-neuron $Ca^{2+}$ signaling was sensitive to gap junction inhibitors, suggesting the direct spread of signal from astroglia to neuronal cells. As such, gap junctions have not been demonstrated in vivo; this result can be considered an artifact of the culture system. However, in neuronal-glial cocultures from visual cortex (329), hippocampus (166), and forebrain (59), glial $[Ca^{2+}]$, waves induced by mechanical (59, 166), electrical (166), or agonist (BK; Ref. 329) stimulation resulted in $Ca^{2+}$ signals in neurons. In these experiments, glial-to-neuronal $Ca^{2+}$ signaling was sensitive to ionotropic GluR antagonists, suggesting a primary role of glutamate released from glial cells in this form of signaling.

Therefore, glial $Ca^{2+}$ signaling is involved, at least in culture systems, in bidirectional neuronal-glial signaling. The questions of the physiological role of these signals, their precise cellular mechanism, and their occurrence in the intact nervous system remain unanswered and deserve further study.

VIII. GLIAL CALCIUM AND BRAIN PATHOLOGY

It is a well-established paradigm that cellular $Ca^{2+}$ overload is a key trigger in causing cell death (66). Much is known about $Ca^{2+}$ toxicity in neurons; however, the problem of $[Ca^{2+}]$, damage to glial cells is much less explored. Experiments on cultured cells demonstrated that $Ca^{2+}$ overload of Schwann cells (291) and oligodendrocytes (29, 377) might severely damage or kill them. In neurons exposed to hypoxia or ischemia, the $Ca^{2+}$ overload results mainly from overactivation of GluRs produced by excessive glutamate release (glutamate excitotoxicity). Astrocytes but not oligodendrocytes are generally believed to be more resistant to excitotoxic insults; astrocytes can tolerate a very high (up to 5 mM) glutamate concentrations, whereas oligodendroglia die when challenged with 200 $\mu$M glutamate (326). This result is probably because the most abundant astrocytic ionotropic GluRs are of AMPA type, characterized by rapid desensitization. Removal of AMPA receptor desensitization by cyclothiazide dramatically increased astrocytic vulnerability to glutamate (93). The high resistivity of astrocytes to glutamate coincides with their role as a major glutamate sink in the brain; it is well established that astrocytes are mainly responsible for glutamate removal from the extracellular space (212), being thus one of the important components of nerve tissue defense against glutamate excitotoxicity.

Injury or brain pathology leads to a complex reaction from the astrocytes and microglial cells that are an important part of the brain’s defenses (145, 252). Astrocytes respond with a variety of biochemical, structural, and proliferative changes that transform them into so-called “active astrocytes.” Microglia also undergo dramatic morphological and biochemical changes transforming them into phagocytic macrophages. The initial signals producing these long-term changes are not well understood; there is some evidence that $Ca^{2+}$ signaling is an important (or maybe even triggering) element in the glial response to these brain insults. Studies in both in vitro and in vivo experimental models used to study ischemic damage revealed that brief exposures of astrocytes to hypoxic/hypoglycemic conditions trigger a $[Ca^{2+}]$, increase as a result of both activation of voltage-gated $Ca^{2+}$ channels and $Ca^{2+}$ release from internal pools (105, 169). The ischemia-induced $[Ca^{2+}]$, increase was activated either by a propagating wave of raised $[K^+]_o$, accompanying injury-induced spreading depression, or by a massive release of neurotransmitters. Interestingly, $[Ca^{2+}]$, was much more sensitive to ischemia in astrocytes in situ, in hippocampal slices, than in the same cells acutely isolated (105). This suggests that the ischemia-induced increase in $[Ca^{2+}]$, in astroglia results from factors released by the damaged tissue (e.g., excess of neurotransmitters or elevated $K^+$ concentration). Intracellular $Ca^{2+}$ increases in astrocytes induced by brain damage might change the functional state of the cell and lead to the release of growth factors or result in astrocytic volume changes.

Another potential pathological $Ca^{2+}$ signal in glia is triggered by the human immunodeficiency virus-1 envelope protein gp120. It induces $[Ca^{2+}]$, increases in both cultured astrocytes and oligodendrocytes from cerebellum (76) and cortex (77), but not in neurons (72). These $[Ca^{2+}]$, responses varied between single spikes and $[Ca^{2+}]$, oscillations. An $[Ca^{2+}]$, elevation was also observed in cultured astrocytes attacked by a fragment (so-called PrP 106–126) of another pathogenic protein, infectious prion protein. This $[Ca^{2+}]$, increase was prevented by the dihydropyridine $Ca^{2+}$ antagonist nicardipine or by $Ca^{2+}$ removal from the incubation media, suggesting the leading role of $Ca^{2+}$ influx through voltage-gated channels (130).
There have been only a few studies of changes in oligodendrocyte \([\text{Ca}^{2+}]\), after brain damage. A possible link between \([\text{Ca}^{2+}]\) in oligodendroglia and brain pathology was suggested by experiments demonstrating that the addition of complement triggered \([\text{Ca}^{2+}]\), oscillations in primary cultured oligodendrocytes (446). These oscillations were blocked by thapsigargin, indicating an important role of intracellular \([\text{Ca}^{2+}]\) stores in this phenomenon.

Microglia detect and respond to brain pathology (246). Damage to the CNS triggers a complex cascade leading to a transformation of microglial cells from a resting form to an active form, termed a macrophage (145). The mechanisms of \([\text{Ca}^{2+}]\), homeostasis and the role of \([\text{Ca}^{2+}]\) signaling in microglial function are not known in detail. Microglial cells do not respond to the “classical” neurotransmitters such as glutamate or GABA. They, however, are responsive to signaling molecules from the immune system such as cytokines or chemokines. For example, activation of complement receptors C5a or C3a triggers a \([\text{Ca}^{2+}]\) increase in cultured microglial cells (292, 318). The microglial [Ca\(^{2+}\)], transients were also observed in response to extracellular application of lipopolysaccharide, a potent activator of immune cells (16). The study of the \([\text{Ca}^{2+}]\) signaling in microglial cells is at an early stage: the role of [Ca\(^{2+}\)], in microglia activation remains obscure.

IX. CALCIUM SIGNALS AND GLIAL FUNCTION

Glial cells exhibit various mechanisms for controlling and varying [Ca\(^{2+}\)]. As described above, modulation of a number of intracellular cascades and membrane pathways enables a variety of external stimuli to induce changes in [Ca\(^{2+}\)], the \([\text{Ca}^{2+}]\) signals. Glial cells are able to sense and react to various neuroactive substances; their reactions almost always involve changes in [Ca\(^{2+}\)]. What glial functions are modulated or initiated by such signals? A number of studies indicate that activation of glial \([\text{Ca}^{2+}]\) cascades affects glial K\(^+\) channels (82, 359), thereby modulating ionic buffering properties of the glial synctium. Furthermore, increases in [Ca\(^{2+}\)], promote glycogen breakdown (343) and gene expression (264, 342) and trigger release of neuroactive substances (322, 330). Calcium influx into glial cell precursors triggers a phosphorylation of cAMP response element binding protein (341) that is known to be a key factor in Ca\(^{2+}\)-dependent gene expression. Noradrenaline, which increases [Ca\(^{2+}\)], also regulates the uptake of glutamate and GABA into astrocytes (164). In addition, the propagation of Ca\(^{2+}\) waves through synctial networks of neuroglia via gap junctions can serve to coordinate glial activity and to transfer information between neurons opposed to different parts of the network. This signal transfer can participate in the integrative functions of both glial and neuronal networks.

When a glial cell has multiple receptors that lead to changes in [Ca\(^{2+}\)], can it respond appropriately to each signal? There are experimental results to indicate that a single cell either in tissue culture (128) or in situ (235, 239, 419) responds to individual ligands with a cell-specific characteristic increase in [Ca\(^{2+}\)], that has a different spatiotemporal pattern. Thus an individual cell may respond to ATP with a brief spike and to norepinephrine or endothelin with an oscillatory or sustained increase in [Ca\(^{2+}\)].

The relative increase may vary in different cellular regions, e.g., processes versus soma, depending on the stimulus and lead to quite different functional responses. Moreover, the exact source of the Ca\(^{2+}\), voltage-dependent channels versus release from the ER, may create large spatial Ca\(^{2+}\) gradients within the glial cell. Therefore, although the results require confirmation, it appears that Ca\(^{2+}\) signaling exhibits the flexibility to enable the cells to respond in an appropriate stimulus-specific manner. These considerations do not rule out the possibility that there are other important intracellular signaling substances in glial cells that have not been well characterized for lack of appropriate detectors. There can be no question but that the ready measurement of [Ca\(^{2+}\)], with fluorescent probes has greatly accelerated the appreciation of the role of this ion in the signaling chain.

X. CONCLUDING REMARKS: CALCIUM SIGNALS ARE A CONSEQUENCE OF GLIAL EXCITABILITY

The classical view that glia are inexcitable refers to their inability to respond to electrical stimulation with a propagated electrical response, i.e., an action potential. However, as documented throughout this review, glial cells are not passive; they display diverse temporal and spatial increases in [Ca\(^{2+}\)], in response to a variety of stimuli, e.g., chemical, electrical, or mechanical. These [Ca\(^{2+}\)], increases may outlast the stimulus and exhibit agonist-specific spatiotemporal patterns, thus providing a possible means for information coding. The glial [Ca\(^{2+}\)], signals are apparently also capable of propagating without decrement via gap junctions into neighboring cells and even to neurons.

The ability of glial cells to actively respond to external stimuli makes them excitable. To avoid confusing this excitability with electrical excitability, it is best to refer to glial cells as “internally calcium excitable.” It may even indeed be appropriate to describe all electrically inexcitable cells that do respond to stimuli via second messengers as “internally second messenger excitable.” As detailed above, there is quite a bit known about the physiology of glial Ca\(^{2+}\) signaling. A beginning has been made in the attempt to characterize in detail the molecular cascades involved in [Ca\(^{2+}\)], homeostasis and shaping of [Ca\(^{2+}\)], signals in glia. The questions that remain to be answered are as follows: How is Ca\(^{2+}\) signaling involved
in the integrative function of glial cells? Is glial Ca\(^{2+}\) signaling involved in information processing in glial networks? Finally, how is glial Ca\(^{2+}\) signaling involved in brain function? These intriguing questions will be the subject of continued study in this area.

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